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OBSERVATIONS ON THE ELASTIC TISSUE OF THE SKIN WITH A NOTE ON THE RETICULAR LAYER AT THE JUNCTION OF THE DERMIS AND EPIDERMIS

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INTRODUCTION

Sternberg (1925) called attention to the fact that there were other tissues besides the skin that showed elastic properties, though not showing any yellow fibres, and conversely, elastic staining tissue did not always show elastic properties. He therefore suggested that the term 'yellow elastic tissue' should be abandoned and replaced by 'yellow connective tissue'. Hass (1939) emphasized that elastic fibres were always associated with collagen fibres, and considered that inferences as to the separate properties of each might be unjustly drawn.

Geiger (1927), studying the development of elastic tissue in the skin, recognized it first in the plantar skin of a foetus 20 cm. long. The elastic tissue of the skin varies in character and amount, both with age and in different parts of the body. Lindholm (1931) found that elastic fibres were finer and more numerous in infants than in adults, and that there was often more elastic tissue in the skin of women than of men. Schmidt (1891) and Kissmeyer & With (1922) described the changes in the skin in old age and after exposure to the weather, and Ohno (1925) in various pathological conditions.

All these workers studied only limited areas, and the present paper describes the character and arrangement of the elastic tissue in skin over almost the whole body. Its appearance will be described as seen in the following areas:

Foot: various parts of the sole, and centre of the dorsum.

Limbs: midway along the legs, thighs, forearms and arms, from the medial and lateral sides.

Abdomen: midline, 2–3 in. above the umbilicus.

Chest: over the junction of the manubrium and body of the sternum.

In addition, skin was examined from subjects with oedema of various types in order to investigate the characteristics of the yellow elastic fibres compared with those of normal skin.

Small elastic fibres were found to approach close to the bases of the deepest layer of epidermal cells. In order to find out whether the elastic tissue takes part in the junction between the dermis and epidermis, investigation of the reticular layer between these tissues was also undertaken.

MATERIALS AND METHOD

The age groups chosen and the number of cases in each group in which all the above sites were examined were as follows:

	Males	Females	Total
(1) 0– 5 years	4	3	7
(2) 15–23 years	4	5	9
(3) 30–40 years	5	3	8
(4) Over 65 years	4	4	8
Totals	17	15	32

Skin from other situations, e.g. scalp, eyelids and eyebrows, back, elbow, knee and scrotum, was examined in some of these cases and from numerous other subjects where suitable material was available.

Small, rectangular portions of skin and subcutaneous tissue were excised, transversely and parallel to the long axis of the body, from the situations mentioned above. These were fixed in a saturated aqueous solution of corrosive sublimate, embedded in paraffin and cut at right angles to the skin surface, in the long axis of the rectangular strip. All sections were stained with haemalum and eosin, and by Weigert's method for elastic tissue, using carmalum or safranin as counterstain. To study the reticular fibres, Foot's (1929) silver impregnation method was also used on sections from many of the blocks; in order to get reasonable impregnation of the subepithelial fibres, it was found necessary to leave the sections rather longer in the silver bath than was required to show the reticular fibres elsewhere (e.g. kidney, aorta, glands), with the result that there was slight over-impregnation, so that the connective fibres in the rest of the dermis were stained deeply; various counterstains were used, safranin proving the best.

In a few cases small blocks from various situations were cut in serial sections, parallel to the surface.

Since the skin contracted when incised, the following experiment was carried out to prevent this. On a thin cork, a circle 1 cm. in diameter was drawn and eight fine needles were pushed through the cork round this circle so that their sharp points projected about $\frac{1}{2}$ cm. on the other side. These were pushed well into the intact skin which was then excised with at least 1 cm. around the points and, with the needles still in position, the specimen was then fixed in corrosive sublimate. On histological examination thereafter, the part within the needle points showed no obvious difference in the elastic tissue from sections prepared in the ordinary way.

RESULTS

A. Normal skin

The elastic tissue in the normal skin consists of a superficial subepidermal layer of fine fibres forming a plexus, and of a deeper layer of much larger fibres (Figs. 5, 9*). The latter seem to be the more important in connexion with the

* Plates 1–4 contain Figs. 1–28.

gross physical property of elasticity in the skin. The quantity and arrangement of the elastic tissue was very similar in the same area of different individuals in the first and last age groups, but showed considerable individual variation in the middle age groups (15–40 years).

Deeper layer of main elastic fibres

This consists of large fibres of considerable length, arranged nearly parallel to the surface of the skin: the fibres are slightly tortuous, but lengths of 200μ or more are seen in sections 8μ in thickness (Figs. 1, 5 and 8). They run between the white collagen fibres, either transversely or parallel to the long axis of the body but not obliquely. In some areas (e.g. lateral side of the thigh, Fig. 3), they are distributed evenly throughout the thickness of the dermis, in others (e.g. medial side of the forearm, Fig. 5) they are more numerous or larger in the superficial part, or in the deeper (medial side of the leg, Fig. 1), or they may be arranged in bundles (lateral side of the leg, Fig. 2). The majority of fibres may run in one direction superficially and at right angles more deeply (abdomen, Fig. 7). They sweep round hair follicles and sweat glands (Figs. 1, 5, 10 and 11), leaving the immediate vicinity of these structures free from elastic tissue.

In some areas, most of the fibres are arranged in a definite pattern. Thus, on the medial sides of the limbs, most of them run transversely to the axis of the limb (Figs. 1, 5 and 9). On the lateral side (Figs. 2, 3) there are equal numbers running transversely and longitudinally. These features were constant for these areas in all subjects examined, though Cox (1941), in his study of the cleavage lines of the skin, found that for the most part the connective and elastic tissue fibres ran parallel to the main axis of the trunk and limbs. In most areas, the direction of the fibres varies in different subjects. A few areas show distinctive arrangements of the fibres and these are described later.

Between the skin and deep tissues, over most of the trunk and limbs, there run, through the subcutaneous fat, bands of connective tissue consisting of collagen and elastic fibres (Fig. 2). They allow of considerable range of movement of the skin in the living subject. In the palm and sole, however, the subcutaneous fat is largely replaced by dense connective tissue, composed chiefly of white fibres, which binds the skin firmly to the deep tissues. This alteration in the deep connexions is of importance in preventing the development of oedema in the sole or palm, any oedematous fluid being transferred to the dorsum of the foot or hand respectively, as is found in septic conditions.

Lindholm (1931) found slightly more elastic tissue in the skin of women than in that of men, but in the present series there was little evidence of sexual difference.

(a) Variations for age and site

(1) *First age group* (*0–5 years*). In this age group, the elastic fibres are always numerous, well formed and large (Figs. 1–4 and 11). The skin of the leg, though much thinner than that of the thigh, contains as much elastic tissue, and the lateral side of the limbs shows thicker skin than the medial, but the same

proportion of elastic fibres. On the lateral side of the leg (Fig. 2) there is a tendency for the elastic tissue to be arranged in alternating bands of longitudinal and transverse fibres. The skin from the lateral side of the thigh (Fig. 3) is as thick as that from the abdomen (Fig. 4), but contains a very much greater proportion of elastic fibres. On the abdomen and chest the skin is thick, but the elastic fibres are fewer, smaller and finer than in the limbs.

(2) *Second age group* (15–23 *years*). The skin in all areas is very much thicker than in the children, but the relative variation in thickness of the different sites is maintained. The elastic fibres are large and well formed but fewer than in earlier life (Figs. 5, 6), though the same relative proportions in the different sites are found.

(3) *Third age group* (30–40 *years*). The elastic fibres (Figs. 7, 8) are fairly numerous but show slight irregularity in size compared to the previous groups. They may be evenly distributed or arranged in irregular bundles, leaving small areas bare of elastic tissue. Otherwise, for the different sites, variations in thickness of the skin and in the proportion of elastic fibres are the same as for the previous age group.

(4) *Fourth age group* (*over* 65 *years*). The relative variation in thickness of the skin from the different parts remains the same. In the elastic fibres, degenerative changes are prominent (Figs. 9, 10); these consist of rough thickenings on the fibres, irregular fragmentation of the ends and variety in length of the fibres. Also, in contrast with the even distribution of elastic fibres of younger subjects, there is condensation and aggregation of the fibres into irregular masses.

In skin which is wrinkled and pigmented, as is most commonly found in parts exposed to the weather, such as the face and back of the hand and forearm (Fig. 10), the elastic tissue is increased in amount and irregularly distributed, being condensed into large, solid masses superficially, though the fibres remain separate in the deeper parts (vide eyebrow, Fig. 12).

(*b*) *Variations in special areas*

The number, size and arrangement of the elastic fibres in certain regions of the body merit special consideration, as there are some peculiarities in their pattern.

On the plantar surface of the toes, on the sole and on the palm, there is almost complete absence of the main elastic fibres, only a few running parallel to the surface between the bundles of white fibres. A few fibres are present alongside blood vessels or ducts of sweat glands, i.e. obliquely or perpendicularly to the surface. In the subcutaneous tissue there are larger collections of elastic fibres, arranged in bands.

The greatest number of large elastic fibres is found in the perineum, around the anus, being particularly abundant in the young (Fig. 11). The scrotum possesses practically no elastic tissue—even the fine subepidermal plexus is very poorly developed. Numerous rather short fibres are present in the skin of the penis, running chiefly transversely.

The skin of the back is very thick and contains numerous, large, elastic fibres; the deep surface of the dermis here shows a serrated edge, but the parts projecting into the subcutaneous adipose tissue do not contain so much elastic tissue as do the superficial parts.

Though the skin in the forearm and upper arm is thinner than in the leg and thigh, there is a corresponding proportion of elastic tissue in both limbs.

Although there is a great increase in the number of hair follicles in the scalp, breaking up the bands of connective tissue, yet elastic fibres are present in large numbers, especially in the more superficial part of the dermis.

The eyelids and eyebrows provide a curious contrast in their elastic tissue content. The eyebrows (Fig. 12) are similar to other parts of the face, as described by Ohno (1925), in that there are large collections of elastic tissue alternating with areas in which no elastic fibres are seen; the tissue is condensed into masses, staining deeply with Weigert's stain and situated immediately below the epidermis. The elastic tissue of the eyebrows is subject to excessive degenerative changes occurring with advancing age and exposure. In the eyelids (Fig. 13), however, there are only traces of elastic tissue in relation to hair follicles, glands and blood vessels.

Subepidermal plexus of fine elastic fibres

(*a*) *Age changes*

In the skin of children under 5 years (Figs. 1–4 and 11), in contrast to the abundance of main elastic fibres, there is almost complete absence of any fibres or fibrils immediately under the epidermis. A few small fibres may be seen about the age of 1 year, but there is nothing comparable to the appearances seen in the next age group.

At about 20 years of age (Figs. 5, 20), a very different picture is seen. There is a well-developed plexus consisting of a few small fibres running horizontally, obliquely and sometimes vertically a short distance below the epidermis, and from these finer fibrils spread out towards the epidermis, their number and arrangement varying in different parts of the body. In subjects of 30–40 years (Figs. 7, 15) the plexus is again well developed. In those over 65 years the small fibres are thicker than in younger subjects and the fine fibrils also, as was found by Lindholm (1931). In other cases they do not show these age changes, and occasionally they are completely absent (cf. Figs. 10, 16 and 19).

Around the hair follicles in their course through the dermis the fine plexus follows the indentations of the epidermis which surrounds the hair sheaths. The plexus in this situation shows the same changes for the different ages as elsewhere (Figs. 1, 5 and 10).

(*b*) *Different sites*

The plexus of fine elastic fibres and fibrils varies considerably even in the same section, especially in relation to the dermal papillae. Thus, between the papillae, only a narrow line of small fibres runs parallel to, and a little deeper

than, the bases of the epithelial cells, with a few fine fibrils running at right angles towards these cells (Figs. 5, 15). In the papillae, however, the plexus opens out and characteristic forms for skin from different parts of the body can be determined.

In skin from the sole of the foot, that covering the arch shows practically no fine plexus, whereas that of the ball of the great toe and of the heel shows a very well-developed elastic plexus with closely packed fibres and fibrils in the papillae and at their bases (Figs. 14, 16); this variation is probably due to the varying stress put on the parts in walking. On the dorsum of the foot, in the skin papillae, which occur in groups of two or three separated by stretches of level epithelium, a digitate appearance is found in the fine plexus, with a line of small fibres forming a regular arc of a circle parallel to the epidermis, and from this a few single, long, thin fibrils run out towards the epidermis; between the papillae there is little fine plexus.

In the plexus on the medial and lateral sides of the leg, the fibres on the former are thinner and situated farther from the epidermis than those on the latter; the fibrils on both sides are few and short. On the medial side of the thigh the papillae are large, numerous and rectangular; the small fibres of the plexus are grouped at the bases of the papillae, and the fine fibrils run out in long wavy bundles towards the superficial angles and apex. The fibres on the lateral side of the thigh (Fig. 19) are thicker and form a basal layer some distance from the epidermis with loops of varying length from which a few long, thin fibrils run towards the epidermis.

The structure of the fine elastic plexus in skin from the hand, forearm (Fig. 15) and upper arm corresponds closely with that from the foot, leg and thigh respectively. The few fibres and fibrils found in the skin from the abdomen are shown in Figs. 6 and 7.

Parts of the skin of the back are drawn into a large number of small folds with crushed papillae and epidermal cells. In these papillae (Fig. 17) there is a large amount of fine plexus in which small fibres run up and seem to end by branching into several long, thin fibrils stretching towards the epidermal cells. In other parts of the back (Fig. 18) the fine plexus appears as in the lateral side of the thigh.

The fine elastic plexus becomes greatly enlarged, and the fibres more numerous in connexion with the origin of the arrectores pilorum (Fig. 20). The fibrils extend close up to the epidermal cells and are spread out over a considerable area in relation to each muscle, thus accounting for the dimpling or 'gooseflesh' appearance when the muscles contract to erect the hair on exposure to cold.

Although this fine plexus of elastic tissue is present in the papillary layer of the dermis, neither the small fibres nor the fine fibrils ever come into direct contact with the epidermal cells. In a careful study of over one thousand thin sections of skin taken from different parts of normal subjects, I never found these fine elastic fibrils actually entering the epidermis, confirming the findings

of Schmidt (1891) and Kissmeyer & With (1922), although they did seem to do so in thick sections from the sole of the foot. There is always a distinct space separating the epidermis from the fine elastic plexus. Yet, if an attempt is made to separate the epidermis from the dermis, the usual result is that the epidermis comes away along with the superficial layer of the dermis, containing the fine plexus. Thus, the fine plexus would appear to play some part in binding firmly together the epidermis and dermis.

B. Skin in oedema

The histological changes in the skin were studied in cases of general oedema associated with cardiac and renal disease as well as in localized types due to chronic inflammatory conditions. Changes in the amount and character of the elastic tissue depend on the duration of the oedema rather than on its type. In recent oedema, even though there is a considerable amount of oedematous change throughout the cutis vera (as seen in haemalum and eosin sections), the main fibres conform to the general appearance for a normal subject of corresponding age, except for slight separation of the fibres. In long-standing oedema, however, the large fibres are shortened and broken up, often to a marked degree (Fig. 21). The fine plexus is usually little altered in character but lies much deeper to the epidermis than in a normal case (Fig. 22). This finding is constant for all areas of the body and suggests that the loose areolar tissue immediately below the epidermis collects more fluid in oedema than the denser part with the main white and yellow fibres.

C. Junction between dermis and epidermis: reticular fibres

It was believed at one time (Herxheimer, 1916; Born, 1921; Cowdry, 1934) that there was a definite, continuous, eosinophil basement membrane on which the epidermis rested and which separated it completely from the dermis. With the introduction of the silver impregnation methods of staining, however, a network of reticular fibres was found (Frieboes, 1920; Pautrier & Woringer, 1930), forming a close connexion between the basal cells of the epidermis and the superficial part of the dermis. This subject has been reviewed by Manganotti (1930) who concluded that the reticular layer, i.e. the outermost part of the dermic mesenchyme, did not constitute a formed membrane between the epidermis and dermis but was probably a morphological appearance which the intercellular colloids assumed in relation to the epidermal cells. Szodoray (1931) has described this reticular network in different parts of the skin and shown how the 'lattice fibrils' interweave with the flabelliform processes at the bases of the epithelial cells.

It has already been shown in this communication how the fine elastic plexus forms a network in the papillary layer of the dermis and yet never comes into actual contact with the epidermal cells; there is always a distinct space separating them. The layer of reticular fibres must occupy part or all of this space, and was studied in sections from all the different sites on the body stained by the silver impregnation method.

The most distinctive appearances of this reticular layer were found in the sole of the foot. At the bases of the papillae the reticular tissue consists of numerous very small fibrils, varying in shape and size, set close up to the bases of the epithelial cells and occupying the areas between the downwardly projecting processes of these cells. At the apices of the papillae, however, the reticular fibres are much more distinctive and are often seen very clearly in oblique sections of the tips (Figs. 23–25); here, long wavy fibrils are found just below the epidermis, with short, blunt ends (the so-called 'Homma's bodies'; cf. Homma, 1922) reaching towards the basal cells. Whether the fibrils form an open meshwork spread round the tips of the papillae, into the spaces of which the bases of the epithelial cells fit, or whether they form a series of fibrils running up to the cells and fixing them, either by entering them or by filling the spaces between their basal processes, is difficult to determine. In the lower power view (Fig. 23), bundles of distinct fibrils are seen to spread out slightly at the tips in a palisade arrangement.

A striking 'hedgehog' appearance (Fig. 27) was obtained on the medial side of the leg from a section parallel to the surface, cutting hair follicles transversely; the reticular fibres are arranged in a radiate fashion around the follicles, as if holding them in place. The skin of the posterior surface of the elbow shows little fine elastic plexus; with reticulum staining, however, an impressive arrangement of tall, cylindrical rods was found, in pairs or small clumps, fitting closely into spaces between the epidermal cells at the tips of the papillae with few reticular fibres at the bases of the papillae (Fig. 28). In other parts of the body the reticular layer shows similar evidence of fibril formation in the papillae, and elsewhere appears as an irregular line between the dermis and epidermis (Fig. 26).

Thus, comparison of the photographs of skin stained for reticular fibres with those stained for elastic tissue demonstrates that these kinds of tissue have very different arrangements and distribution and are quite distinct from each other. There is, as far as these staining methods show, no continuation of the fine elastic fibres as reticular fibrils up to the bases of the epithelial cells. Although the fine elastic tissue develops such characteristic forms as have been shown, close under the epidermis, it does not take part in the ultimate junction between dermis and epidermis and can play the part only of a reserve force in preventing separation. The elements concerned in the actual binding together of dermis and epidermis are the reticular layer and the bases of the epithelial cells. In agreement with Manganotti (1930), the results show that the reticular layer is probably a disposition of the intercellular colloids, but, in addition, there is possibly a more definite formation of fibrils in the parts where the reticular network is as well developed as in the tips of the papillae in the sole of the foot.

SUMMARY

The distribution of yellow elastic tissue in the skin is described and illustrated from an investigation of thirty-two normal subjects with additional observations from other subjects.

The elastic tissue consists of two parts:

(i) large fibres in the deeper part of the dermis,

(ii) a fine network of small fibres lying close under the epidermis.

Variations in the quantity and characteristics of these two parts are described and illustrated in:

(i) skin from different situations of the body,

(ii) skin from subjects of different ages.

A brief description is given of the changes in the elastic tissue in subjects with oedema.

The reticular fibres of the skin at the junction of dermis and epidermis have been shown to be distinct from the elastic tissue.

This work was carried out during the author's tenure of the Hutchison Research Scholarship, 1934–5 and thereafter under the kindly guidance of the late Prof. J. Shaw Dunn.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Skin of leg, medial side, transverse. Main elastic fibres large, well formed and distributed fairly evenly, though larger in the deeper parts and absent round hair follicles (*A*). 15843, male, 1 year. Weigert's elastic tissue stain, ×40.

Fig. 2. Skin of leg, lateral side, transverse. Main elastic fibres arranged mostly in bands. *A*, subcutaneous band of connective and elastic tissue. 15843, male, 1 year. Weigert, ×40.

Fig. 3. Skin of thigh, lateral side, transverse. Main elastic fibres numerous and evenly distributed. 4188, male, 1½ years. Weigert, ×40.

Fig. 4. Skin of abdomen, transverse. Main elastic fibres shorter and fewer than in thigh (Fig. 3). 4188, male, 1½ years. Weigert, ×40.

Fig. 5. Skin of forearm, medial side, transverse. *A*, main elastic fibres, most numerous towards the superficial part of the cutis vera, and mostly running in line of section. *B*, fine elastic plexus. 15665, female, 17 years. Weigert, ×40.

Fig. 6. Skin of abdomen, longitudinal. Main fibres few and short, distributed evenly through skin 16088, male, 15 years. Weigert, ×40.

Fig. 7. Skin of adbomen, longitudinal. Main fibres relatively short and thin, arranged parallel to line of section near the surface, transversely deeper. 16110, female, 35 years. Weigert, ×40.

Fig. 8. Skin of thigh, medial side, transverse. Main fibres large, but slightly irregular in shape and distribution. 16110, female, 35 years. Weigert, ×40.

Plate 2

Fig. 9. Skin of thigh, medial side, transverse. *A*, main fibres, more irregular in size and distribution than in Fig. 8. *B*, fine elastic plexus. 15785, male, over 65 years. Weigert, ×40.

Fig. 10. Skin of forearm, lateral, side, transverse. Elastic tissue irregularly thickened and condensed—degenerative changes due to old age and exposure. 15795, female, 77 years. Weigert, ×40.

Fig. 11. Skin of perineum. Main elastic fibres are very numerous, except round sweat gland (*A*). 15843, male, 1 year. Weigert, ×40.

Fig. 12. Skin from eyebrow. Note large quantity of dense elastic tissue under the epidermis. Accident case, male, about 35 years. Weigert, ×40.

Fig. 13. Eyelid—skin surface above, conjunctival surface below. No elastic tissue present except round hair follicles and in vessel walls. Same case as Fig. 12. Weigert, ×10.

Fig. 14. Skin of sole of foot, transverse. Dense plexus of fine elastic fibres in several papillae. A6, male, 70 years. Weigert, ×150.

Fig. 15. Skin of forearm, lateral side, transverse. Small elastic fibres very distinct. 16110, female, 35 years. Weigert, ×600.

Fig. 16. Skin of sole of foot, transverse. Typical appearance of fine elastic plexus in this site. 15834, female, 64 years. Weigert, ×600.

Plate 3

Fig. 17. Skin of back. Folded part of skin with fine elastic plexus of small fibres crushed together. 15834, female, 64 years. Weigert, ×600.

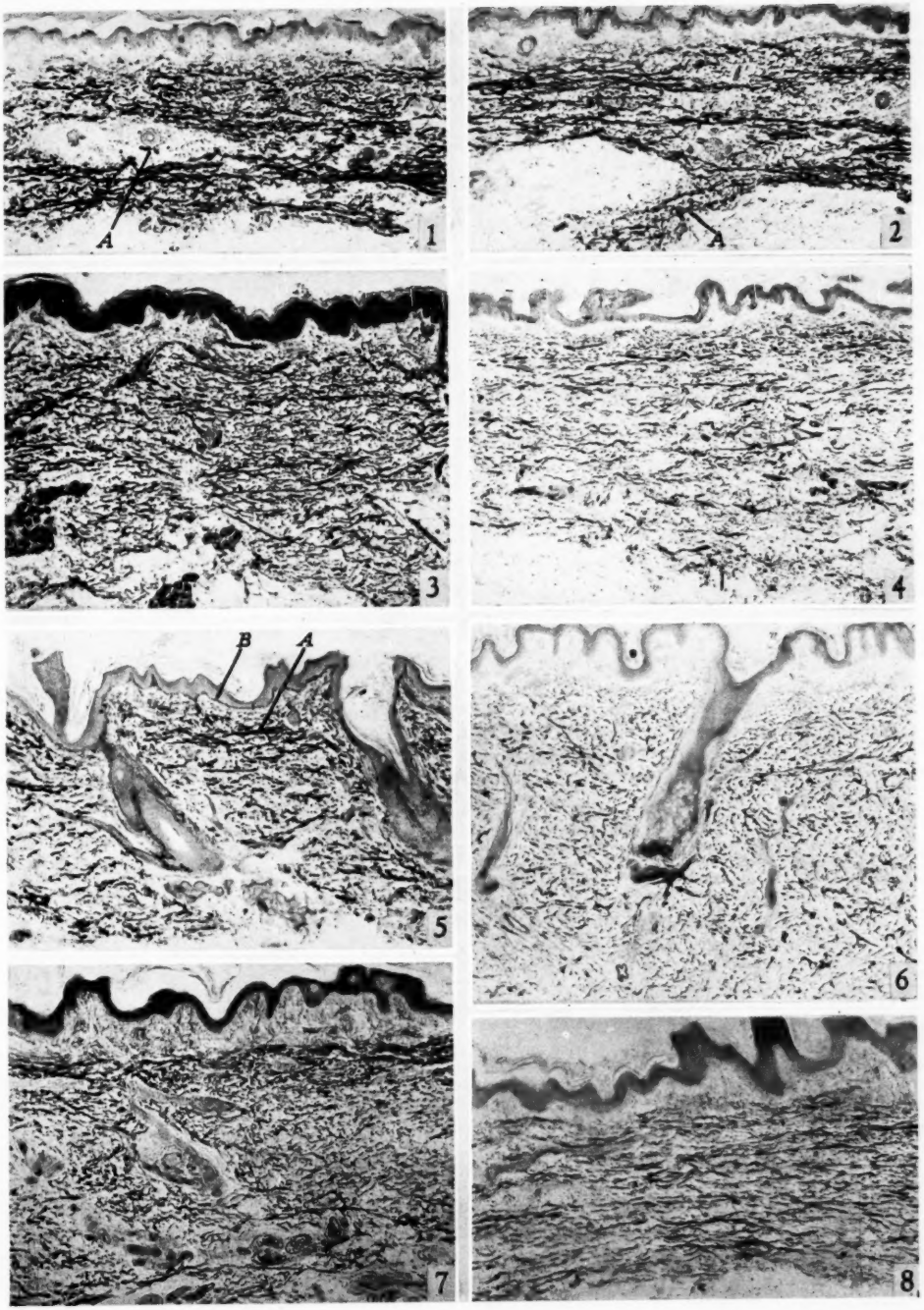
Fig. 18. Skin of back (cf. Fig. 17). Part of skin with no fold: fine elastic plexus spread out. 15834, female, 64 years. Weigert, ×600.

Fig. 19. Skin of thigh, lateral side, longitudinal. Small elastic fibres and fibrils under epidermis. 15834, female, 64 years. Weigert, ×600.

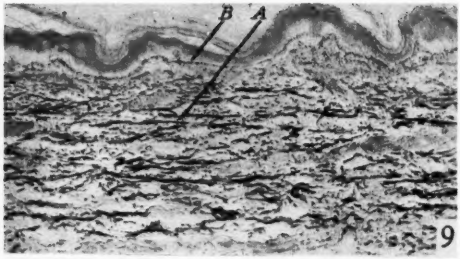
Fig. 20. Skin of arm, lateral side, transverse. Elastic tissue well developed at origin of *arrector pili* (*A*). 16073, female, 23 years. Weigert, ×150.

Fig. 21. Skin of abdomen, longitudinal. Oedema. Main fibres slightly separated. Fine plexus some distance from epidermis. 16140, male, 70 years. Weigert, ×150.

Fig. 22. Skin of abdomen, longitudinal. Oedema. Fine elastic plexus set well below epidermis. 16140, male, 70 years. Weigert, ×600.



DICK—ELASTIC TISSUE OF THE SKIN



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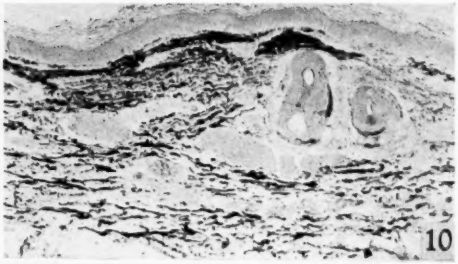

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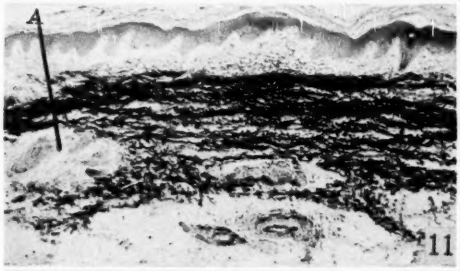

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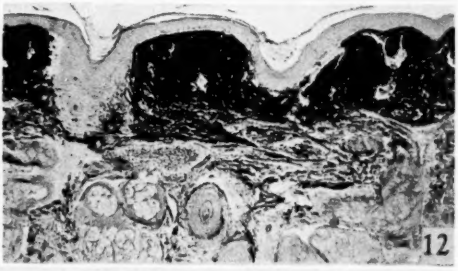

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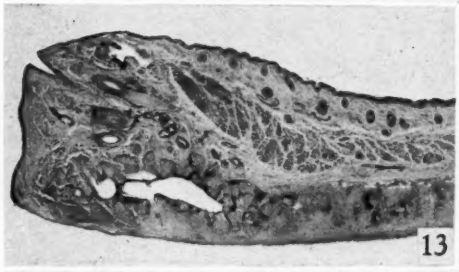

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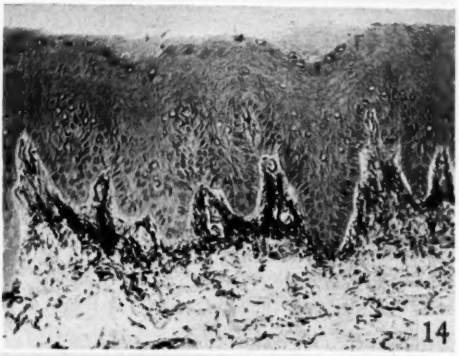

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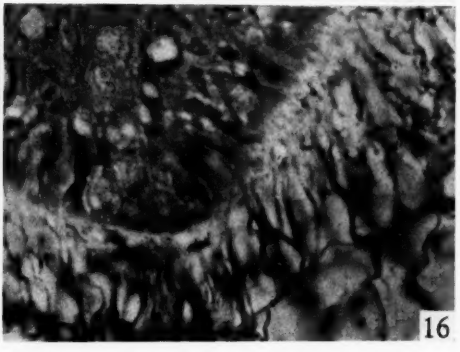

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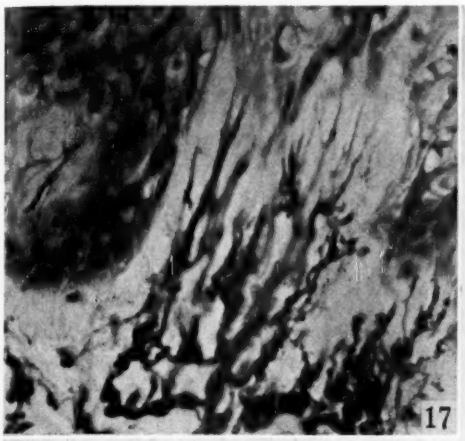
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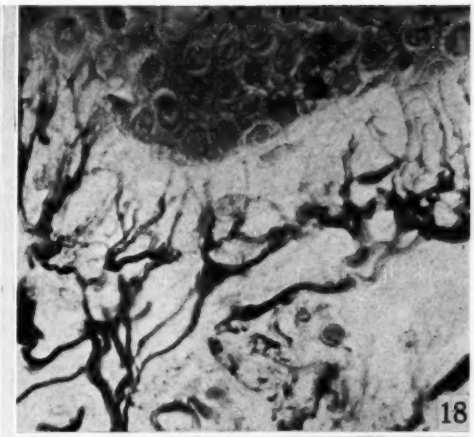
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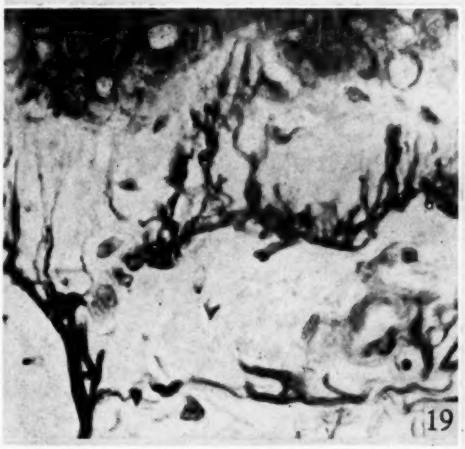
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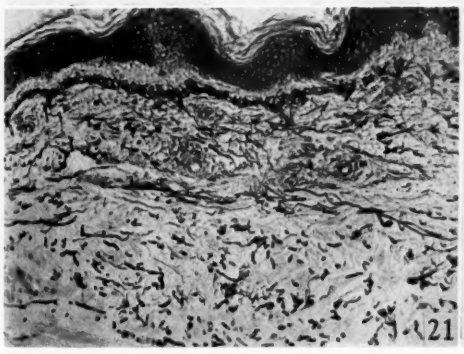
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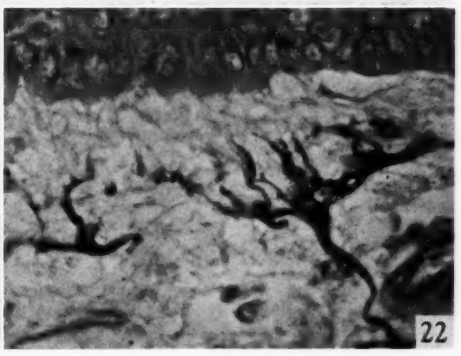
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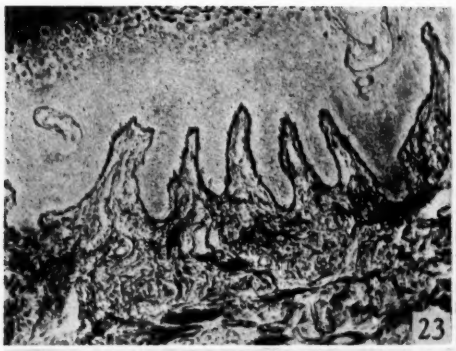

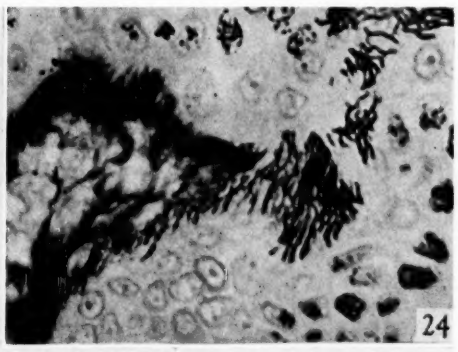

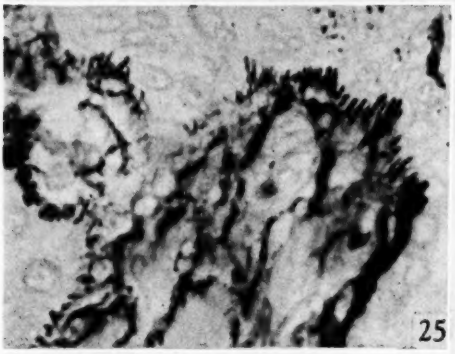

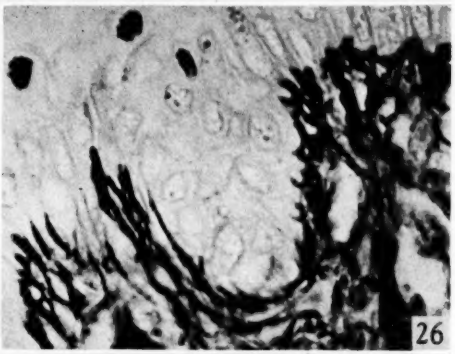

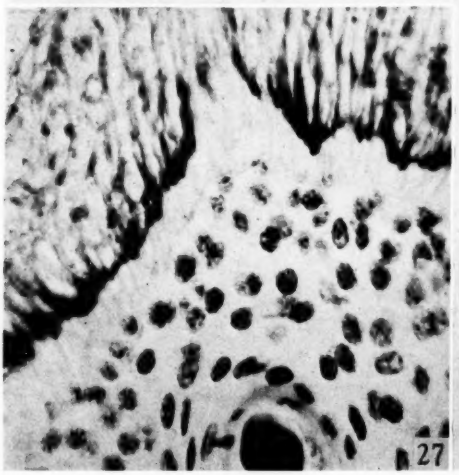

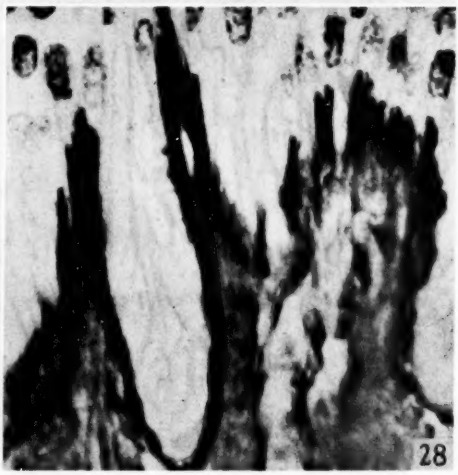

DICK—ELASTIC TISSUE OF THE SKIN

PLATE 4

Fig. 23. Skin of sole of foot. Several papillae with reticular fibres at their tips. A 6, male, 70 years. Foot's silver impregnation method, × 150.

Fig. 24. Skin of sole of foot. Reticular fibrils long and wavy, forming a loose network, in oblique section, near the tip of a papilla. A 2, male, 31 years. Foot, × 600.

Fig. 25. Skin of sole of foot. Reticular fibrils forming definite rods (Homma's bodies) at tip of papilla. A 2, male, 31 years. Foot, × 600.

Fig. 26. Skin of dorsum of foot. Reticular tissue indefinite at bases of papillae, more definitely rod-shaped at tips. A 2, male, 31 years. Foot, × 600.

Fig. 27. Skin of leg, medial side, cut parallel to the surface. Note distribution of reticular fibrils round hair follicle. A 2, male, 31 years. Foot, × 600.

Fig. 28. Skin of elbow, posterior surface. Formation of definite reticular fibrils at tips of papillae. 15682, male, 17 years. Foot, × 600.

EXPERIMENTAL FOETAL DEATH IN THE RAT: HISTOLOGICAL CHANGES IN THE MEMBRANES

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Departments of Anatomy and Physiology, St Mary's Hospital Medical School

It is generally known that intrauterine death of mammalian foetuses is not necessarily followed by death of the foetal membranes, but so far morphological investigations of the surviving membranes have been chiefly concerned with natural foetal death, and very few studies after artificial death have been made. The interpretation of the histological appearances of the uterus after spontaneous foetal death is made difficult by the fact that the time and cause of death are usually not known, so that it is often impossible to distinguish ante-mortem from post-mortem changes and changes occurring as a direct consequence of foetal death from those due to independent pathological disturbances.

It was therefore decided to kill healthy rat foetuses at known stages of pregnancy by methods which disturbed the membranes as little as possible, and to study the subsequent development of the membranes under controlled conditions.

Preliminary reports of this work have already been published (Huggett & Pritchard, 1945 *a*, *b*). The present paper deals with a larger series of experiments and some of the earlier conclusions have been modified in the light of the more recent work.

MATERIALS AND METHODS

Forty Norwegian rats bred from the Lister hooded strain and aged 6–12 months were used. Foetal destruction was carried out on the 10th and subsequent days of pregnancy up to the 19th; the day after finding sperms in the routine morning vaginal smear was taken as the first day of pregnancy. The 10th day of pregnancy was chosen for operation in a high proportion of the rats for the following reasons: (1) it was found impossible to perform the operation satisfactorily before the 10th day, (2) we particularly wished to study the fate of trophoblast which had never been vascularized from the allantois, and (3) in our strain of rats such vascularization took place either on the 11th day or late on the 10th day.

Under ether anaesthesia, and with aseptic technique, an incision was made in the left flank, and the left horn of the uterus was identified. The foetuses in this horn were destroyed either by crushing them with toothed forceps through the intact uterine wall (10th–12th days) or by removing them through small incisions made in the antimesometrial part of the uterus (13th–19th days). In the former series most, if not all, of the amniotic fluid escaped through the

punctures made by the forceps; in the latter series loss of amniotic fluid was, of course, complete. Bleeding was slight and the uterus was not sutured. The right horn was left intact, as a control. There were no septic cases, and only one animal died as a result of the operation.

The rats were killed at intervals of 1–11 days after operation. Three rats delivered themselves spontaneously before death. After inspecting the uterus, segments containing foetuses and/or membranes were cut from both horns and placed in Bouin's or Zenker's fluid. The smaller uterine swellings were fixed entire, but the larger were opened after preliminary fixation in order to accelerate the penetration of the tissues by the fixatives. Specimens were sectioned serially in paraffin at 4–10 μ and alternate sections were stained with Delafield's haematoxylin and eosin, and Weigert's haematoxylin and Van Gieson, with the exception of a few which were stained for glycogen with Best's carmine.

Since the rat's placenta is approximately symmetrical about an axial artery, the most useful sections were those which included a considerable length of this vessel, because they could be considered as central, and therefore legitimate comparisons of size and shape could be made from them. Appearances were often misleading in sections which did not include this vessel.

TERMINOLOGY

Mossman's (1937) systematic nomenclature for the decidua and foetal membranes of rodents, based on comparative studies, has been used in this paper, but reference has been made also to the work of Duval (1891), Robinson (1904), Goldmann (1912), Huber (1915), Evans & Burr (1927), Grosser (1927), Everett (1935), Selye & McKeown (1935) and Krehbiel (1937).

OBSERVATIONS

One rat died on the day after operation and in two others the foetuses were not destroyed by the crushing operation; in the remaining thirty-seven animals ninety-six foetuses were destroyed. From the appearances in sections it was evident that the foetal membranes corresponding to seventy-five of these had been alive when the uteri were removed 1–11 days later, while in the remainder the foetal tissues had either been completely resorbed, or were represented solely by necrotic debris. Thirteen of these twenty-one cases followed operation on the 10th day of pregnancy and the remainder followed operation between the 15th and 17th days. All occurred among the earlier experiments, when the operative technique was being developed, which suggests that faulty operative technique was responsible for the failure of the membranes to survive. The descriptions which follow are based on sections of the seventy-five uterine swellings which contained living foetal tissues, and on twenty-four normal controls from the undamaged right horns.

Foetal destruction at the 10th day

The chief features of the histology of the normal uterus and membranes at this time are shown in Pl. 1, fig. 1 and Pl. 2, figs. 7, 8. Lining the decidua capsularis may be seen the bilaminar omphalopleure (Pl. 2, fig. 7) consisting of mononuclear giant cells, Reichert's membrane and the parietal entoderm of the yolk sac. Just inside the omphalopleure the inverted wall of the yolk sac, comprising visceral entoderm and vascularized splanchnopleure, is present. Inside this again lie the amnion and the embryo. The placental trophoblast (Pl. 1, fig. 1) forms a conical mass projecting into the decidua basalis. It exhibits three zones of cells (Pl. 2, fig. 8). The outermost is a layer of giant cells similar to, and continuous with, those of the omphalopleure. Next there is a spongy zone of small cells interspersed with maternal blood spaces. The innermost zone consists of a curved plate of closely packed cells at the base of the cone which is fitted over the expanded extremity of the mesodermal allantois. The last-named shows a few small blood vessels but these are only just beginning to invade the curved plate.

Two days after operation (Pl. 1, fig. 2) the embryo, amnion and inner wall of the yolk sac had been reduced to a structureless coagulum occupying a cavity in the remaining foetal tissues, which were still alive as judged by their staining reactions. The cavity was bounded antimesometrially by the omphalopleure and mesometrially by the placental trophoblast and allantoic mesoderm: it therefore represented a coalescence of the yolk sac lumen, exocoelom and amniotic cavity.

The parietal entoderm, Reichert's membrane and the giant cells of the omphalopleure were healthy in appearance and some of the entodermal cells were in mitosis. The giant cells had obviously increased considerably in numbers since the time of operation: they also formed a thicker layer than in the 12th-day controls, but this was more likely due to the fact that they were spread over a smaller surface, than to any absolute superiority in numbers.

The placental trophoblast at the 12th day had also grown considerably since the 10th day (Text-fig. 1). Its three zones were still sharply defined. The giant-cell and spongy zones were thicker than in the 12th-day controls, presumably for the same reason as that given above. Many of the cells of the spongy zone were in mitosis. The curved plate had made an attempt at forming a labyrinth but this was much smaller than normal and was devoid of foetal blood vessels; near the allantois it consisted of compact masses of cytotrophoblast, but adjacent to the spongy zone a syncytial network had been formed (cf. Pl. 3, figs. 10, 11 with Pl. 2, fig. 9).

The allantois (Pl. 1, fig. 2 and Pl. 2, fig. 10) was represented by an island of loose avascular connective tissue at the centre of the foetal surface of the labyrinth.

The decidua basalis was thicker than in the controls, but was healthy in appearance and normal in structure. The decidua capsularis was totally necrotic, and was beginning to slough from the uterine wall. The holes in the

latter made by the forceps at operation were plugged with giant cells from the omphalopleure.

By the 13*th day*, in one specimen, most of the coagulum had been resorbed and the cavity in the foetal tissues was almost obliterated through apposition of its mesometrial and antimesometrial walls (Pl. 1, fig. 3). In another specimen (Pl. 1, fig. 4) the antimesometrial part of the foetal tissues, consisting of the greater part of the omphalopleure, the allantois and some of the labyrinth, was necrotic, though Reichert's membrane could still be distinguished among the debris. The condition seen in Pl. 1, fig. 3 evidently precedes that seen in Pl. 1, fig. 4, for all later specimens from 10th-day operations showed destruction of the omphalopleure and allantois.

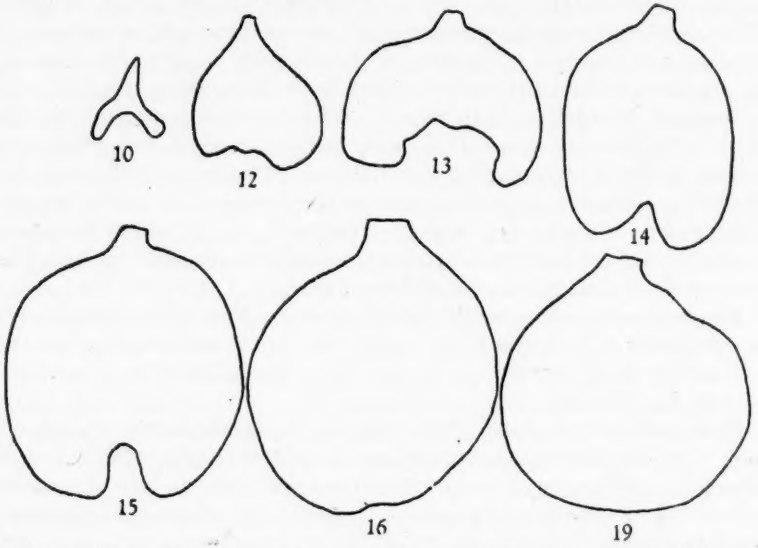
The surviving foetal tissues included the placental trophoblast, and some of the giant cells from the omphalopleure. Together (Pl. 1, fig. 4) they formed a swelling shaped like an inverted cup attached to the decidua basalis over its mesometrial hemisphere, with necrotic debris occupying the interior. The swelling had the appearance of projecting into the uterine lumen, whereas the normal placenta at this stage appears to be embedded in the uterine wall. The trophoblast formed a larger mass than on the previous day, chiefly owing to rapid growth of the spongy zone. The labyrinth was far behind the normal controls in size, but the differentiation of its cellular trophoblast into a syncytial network continued as in normal development.

The decidua capsularis had sloughed from the uterine wall completely. The newly formed uterine epithelium, unlike that of the controls, but like that normally found in between conceptuses and in sterile horns, was thrown into villous folds (Pl. 1, figs. 3, 4 and Pl. 3, fig. 12).

By the 14*th day* the omphalopleure, allantoic mesoderm and the neighbouring part of the labyrinth had become almost completely resorbed. The rest of the labyrinth and the spongy zone continued to grow, while the cells of the latter showed the vacuolation which normally occurs at this time and is indicative of glycogen storage.

At the 15*th day* the surviving foetal tissues had reached the peak of their development (Pl. 2, fig. 5). They formed a placenta-like organ whose spherical shape, however, was in marked contrast to the discoidal one of the normal placenta. At the 10th day the normal placental trophoblast formed a cone 0·5 mm. in diameter at its base and 1 mm. in height. At the 15th day the surviving trophoblast formed a sphere 5·0 mm. in diameter. From these figures it may be calculated that there had been an approximately one-thousandfold increase in volume during these five days. This rapid growth was also apparent when the outlines of central sections of the surviving foetal tissues, taken at daily intervals after operation, were compared with one another and with a section from the 10th-day control (Text-fig. 1). This figure shows that growth was most rapid in the first three or four days after operation, but that it declined as the 15th–16th days were reached, and ceased altogether after that.

At the 15th day the spongy zone showed intense glycogen storage as in the normal controls. The labyrinthine trophoblast was now almost entirely transformed into syncytium (Pl. 4, fig. 16). Except for the absence of foetal vessels and mesenchyme the architecture of the labyrinth was essentially normal (cf. Pl. 4, fig. 15) and although much smaller than the corresponding normal zone at the 15th day, it was nevertheless considerably larger than the curved plate of the 10th day from which it had been derived. The maternal blood sinuses in it radiated from a wide central arterial channel, and drained into tortuous venous channels traversing the spongy zone; the maternal circulation therefore followed the normal pattern.



Text-fig. 1. Outlines, drawn to scale from central sections, of the placental trophoblast at the 10th day of pregnancy (control) and 2, 3, 4, 5, 6, and 9 days after foetal destruction at the 10th day. × 8·5.

Up to this stage growth and differentiation predominated over degenerative changes.

After the 15*th day* degenerative changes predominated. At the 16th day the glycogen cells of the spongy zone began to disintegrate (Pl. 4, fig. 18), and this was followed, on the 17th day, by extravasation of maternal blood into the spaces vacated by them. At the same time the vascular channels of the giant-cell zone became dilated and then thrombosed. The decidua basalis, which had remained healthy, although somewhat thicker than normal, up to the 16th day, began to show a necrosis spreading towards the centre. This was associated with a spreading separation of the decidua from the uterine wall along the line of the circular muscle. The labyrinth was relatively little affected by the degenerative

changes in these neighbouring zones (Pl. 2, fig. 6) and, apart from scattered pycnotic nuclei, the trophoblastic syncytium remained healthy, at least until the 19th day.

At full term (21 *days*) the residual foetal tissues were shed into the uterine lumen as hard spherical pellets about 4 mm. in diameter, composed chiefly of blood clot.

Foetal destruction on the 11*th and* 12*th days*

Normally in our rats the allantoic blood vessels and connective tissue begin to invade the curved plate of trophoblast late on the 10th or early on the 11th day. The connective tissue forms sheaths around the larger foetal vessels, but not around the smaller (Pl. 2, fig. 9), the endothelium of which lies in direct contact with the trophoblast.

The placental masses formed after foetal destruction on the 11th and 12th days differed from those formed after destruction on the 10th day in that the development of the labyrinth in the former was complicated by the persistence for a longer time of entodermal and mesodermal derivatives (Pl. 3, figs. 13, 14). In other respects the histology of the surviving foetal tissues after 11th- and 12th-day operations was not significantly different from that described for the 10th day; in particular a similar pattern of rapid early growth followed later by spreading degeneration was observed.

Within the labyrinth the connective tissue sheaths for the larger foetal vessels persisted throughout pregnancy. The vessels themselves collapsed after expelling their contents, and their endothelium gradually lost its identity and merged with the surrounding connective tissue. The smaller foetal vessels without connective tissue sheaths also collapsed. Their lumina were obliterated and their normally elongated endothelial nuclei became spherical. Cell boundaries were lost, and a syncytium was formed in which large trophoblastic and small endothelial nuclei shared a common cytoplasm (Pl. 4, fig. 17).

The cellular trophoblast between the foetal vessels continued to proliferate and also to undergo its normal transformation into syncytium, so that the labyrinth as a whole increased in size and new syncytium, composed solely of trophoblast, appeared in increasing amounts between the islands of older heterogeneous syncytium. At the end of pregnancy these islands were still present to mark the position of the foetal blood vessels at the time of operation. From their distribution it was evident that the labyrinth had grown evenly throughout, and that it had not increased by transformation of the trophoblast of the spongy zone into syncytium.

Foetal destruction at the 13*th and* 14*th days*

At this time, normally, the labyrinth is well vascularized from the foetal side and the omphalopleure is beginning to send hollow diverticula into the placenta around the major allantoic vessels to form the 'entodermal sinuses' of Duval. Part of the omphalopleure is reflected on to the foetal surface of the placenta to form a supporting membrane. The remainder of the omphalopleure, together

with the decidua capsularis, is normally necrotic at this stage; Reichert's membrane, however, persists for three or four days longer. When it eventually ruptures it retracts to the margins of the placenta and its tangled remains are visible there until the end of pregnancy.

On the 13th and subsequent days the foetuses were *removed* from the uterus, whereas earlier they had been crushed *in situ*. However, neither the presence of resorbing foetal debris in the earlier group nor the incision in the uterine wall in the later group appeared to have any profound influence on the subsequent pattern of development of the membranes, and so the difference in the method of killing may be discounted.

After operations on the 13th and 14th days of pregnancy the labyrinth showed a similar combination of trophoblastic growth and endothelial involution to that described after operations on the 11th and 12th days. The allantoic connective tissue persisted throughout pregnancy, partly as a compact mass at the centre of the labyrinth, and partly dispersed throughout the labyrinth around the remains of large foetal vessels. The entodermal sinuses of Duval, and that part of the omphalopleure which lines the foetal surface of the placenta, persisted until full term, the entodermal cells remaining alive and showing infrequent mitotic figures.

After operations on the 13th day rounding up of the placental trophoblast was similar to that described after earlier operations. But after operations on the 14th day and later the placenta was evidently more resistant to deformation, for the normal discoidal shape persisted until the end of pregnancy.

There was an important difference between the surviving trophoblast of this group and those in which foetal death occurred on the 10th–12th days, in that the giant-cell and spongy zones were not destroyed by haemorrhages and thromboses in late pregnancy. After an initial period of growth followed by glycogen storage the spongy zone showed an orderly and progressive disintegration of its glycogen cells in an essentially normal manner, without disturbing the maternal circulation. As a result, at the 20th day, following 13th-day operations, the 'placenta' differed from that of controls chiefly in the smaller size of the labyrinth and in the absence of foetal vessels, the other tissues being normal. The decidua basalis did not become necrotic towards the end of pregnancy as in previous groups.

Foetal destruction at the 15*th day*

In most respects the surviving membranes were similar to those after 14th-day operations. The central part of the labyrinth, however, showed a type of pathological disturbance which was characteristic of the entire labyrinth after all later operations, and so its description will be postponed.

Foetal destruction at the 16th–19*th days*

Normally the cellular trophoblast of the labyrinth has all been replaced by syncytium at the 16th day, and the subsequent increase in size of the labyrinth

is due to an increase in the number of blood vessels, associated with a thinning of the syncytium already present. According to Mossman (1937), in late pregnancy the syncytium ruptures in many places so that a haemo-endothelial relationship is established in parts of the placenta.

When the foetuses were removed on and after the 16th day further growth of the placentae ceased. The giant-cell and spongy zones remained normal, but the labyrinth showed a degenerative change which was foreshadowed in the 15th-day group, but was quite unlike anything seen after operations on the 14th day or earlier (Pl. 4, figs. 19, 20). After the collapse of the foetal blood vessels, their endothelial nuclei remained elongated and in linear order, in contrast with their rounding up and clumping together in previous groups. In many places the thinner syncytial strands of trophoblast appeared to have broken down. Around the collapsed foetal vessels there had accumulated a thick layer of exudate, containing fibrin strands and isolated maternal red cells. This exudate was separated from the maternal blood stream by syncytium except where the latter had broken down. In some places so much exudate had formed that the maternal blood channels were obliterated, and local necrosis of the labyrinth ensued; elsewhere they remained patent, and both syncytium and endothelium survived until the end of pregnancy.

The spongy zone, giant-cell zone, residual omphalopleure, entodermal sinuses of Duval (Pl. 4, fig. 20) and the allantoic connective tissue were not materially affected by foetal death at this late stage.

CONCLUSIONS AND DISCUSSION

These experiments have shown that in the rat after foetuses had been removed from the uterus, or crushed *in situ*, the amnion and splanchnopleuric wall of the yolk sac rapidly perished and were resorbed, but the placental trophoblast, allantois and omphalopleure survived, at least for a time. Further, the surviving membranes grew and differentiated in a comparatively normal fashion up to the 16th day of pregnancy, departures from normal development being chiefly referable to (1) the abnormal shape imposed on them as a result of the loss of the amniotic fluid, and (2) the obliterative changes in the foetal blood vessels.

Pathological changes of two kinds were observed after the 16th day; the first, which followed operations on the 10th–12th days, consisted of haemorrhages and thromboses in the giant-cell and spongy zones of the placental trophoblast; the second, which followed operations on the 16th–19th days, was a gross oedematous swelling of the labyrinth.

It should be noted that the surviving membranes were those advantageously placed for receiving nourishment from the maternal circulation, and that they included representatives of all three germinal layers.

At full term the surviving placentae had become completely separated from the decidua basalis, and were expelled.

Where growth was observed it was orderly, and the component tissues remained sharply defined; there was no indication of unregulated or neoplastic growth in any tissue after foetal death. Growth, moreover, followed the normal time sequence, being vigorous in mid-pregnancy but becoming progressively less active later. The greater part of the volume increase was due to proliferation of the small trophoblastic cells of the spongy zone. The accumulation of glycogen cells in this zone proceeded normally.

The most unexpected features observed were (1) the formation of a composite labyrinthine syncytium from the trophoblast and the endothelium of the obliterated foetal vessels, and (2) the oedema of the labyrinth after foetal removal late in pregnancy.

That the foetal membranes may survive for longer or shorter periods after either spontaneous or experimental foetal death is generally agreed. Strahl & Henneberg (1902) in the ferret, Fortuyn (1920) in the mouse, and Evans & Burr (1927) in the rat, found, however, that the ectodermal elements persisted longest. In our experiments also the ectodermal elements survived longer than the others after operations on the 10th–12th days, but after that time ectodermal, entodermal and mesodermal elements were equally viable. In the experiments of Evans & Burr death was due to vitamin E deficiency, which caused a failure of mesodermal development beginning even before foetal death (which usually occurred on the 13th day); the other investigations mentioned studied the effects of spontaneous foetal death in cases in which the cause of death was unknown, and it was possible that the membranes were already abnormal at the time of death. Their results, therefore, do not necessarily conflict with those reported in the present paper in cases in which it was reasonably certain that the membranes were normal at the time of operation.

The evidence concerning the capacity of the membranes for growth after foetal death is difficult to evaluate. Giacomini (1892) removed rabbit foetuses at one-third of the way through pregnancy and found that the membranes continued to grow for several days. Meyer (1917), from a study of spontaneous foetal death in sheep and guinea-pigs, concluded that in those species the *early* chorionic trophoblast possessed considerable capacity for independent growth. Mall & Meyer (1921), however, studied abnormal human ova in which the embryos were stunted or absent, and concluded that it was doubtful whether any growth of the membranes had taken place after foetal death. In vitamin E-deficient rats Evans & Burr (1927) found, as we did in our experiments, that the trophoblast continued to grow until the 16th day. Courrier & Gros (1936) found that in the cat the membranes continued to grow for a further three weeks after removal of embryos at the end of the second week of pregnancy. It is probably significant that where growth has been observed, foetal death took place comparatively early in pregnancy. Other factors, however, such as differences in species and in the cause of foetal death, are important in determining the growth activity of the surviving membranes (Huggett & Pritchard, 1945 *b*).

Of the many kinds of degenerative change which previous investigators have described as occurring in the placenta after foetal death, only four are particularly relevant to the discussion of our findings.

(1) Ballantyne (1902) and Novak (1941) in man, and Evans & Burr (1927) and Selye, Collip & Thomson (1935) in the rat, have described the obliteration of foetal vessels in the chorionic villi and labyrinth respectively; but the subsequent formation of a heterogeneous syncytium such as we have described does not appear to have been noted before.

(2) Hertig & Edmonds (1940) studied a large series of abnormal human ova in which the embryos were dead but the membranes remained alive, and found, in a high proportion of them, that the villi were hydropic. They attributed this to a disturbance of osmotic relations across the syncytium after cessation of the foetal circulation.

The swelling of the labyrinth found in our experiments may have had a similar cause. On the other hand, if Mossman (1937) is correct in his view that the syncytium breaks down in many places as a normal event late in pregnancy in the rat, then the swelling could be simply explained as due to the passage of blood plasma through the gaps in the syncytium to occupy the space vacated by the collapsing foetal vessels. We have not so far been able to confirm Mossman's findings, and so we are unable at present to decide which of these hypotheses as to the cause of the oedema is the correct one.

(3) Progressive coagulative necrosis of the placenta from the foetal to the maternal surface has been described by Fortuyn (1920) after spontaneous foetal death in the mouse, and by Urner (1931) following a severe vitamin E deficiency in the rat. That this did not occur in our experiments suggests that the placentae in their cases were directly affected by the pathological disturbances which killed the foetuses, and that the necrosis was not due to foetal death *per se*.

(4) Evans & Burr (1927), in their vitamin E-deficient rats, found haemorrhages and thromboses in the peripheral parts of the surviving placentae similar to those found by us after the destruction of foetuses on the 10th–12th days of pregnancy. The reasons for these disturbances of the maternal circulation cannot be stated with certainty, but it is probably significant that they occurred in placentae in which the giant-cell and spongy zones were abnormally thick, the labyrinth was poorly developed and the decidua was thick and necrotic. In a previous paper (Huggett & Pritchard, 1945 *b*) we stressed the lack of mesodermal derivatives in the labyrinth as a factor predisposing to this breakdown of the maternal circulation, but it now seems more probable that this merely contributes to the poor development of the labyrinth and is not otherwise significant.

Few investigators have discussed the extent to which normal histological differentiation may continue in the placenta after foetal death. In the rat, Evans & Burr (1927) and Selye & McKeown (1935) described the formation of islands of glycogen cells in the spongy zone, and the former workers described the transformation of the cellular trophoblast of the labyrinth into a syncytial

network enclosing wide maternal sinuses; both of which observations we have confirmed.

Evans & Burr were struck, as we were, with the increased thickness of the giant-cell zone as compared with normal controls, and the fact that this appeared to be inversely related to the size of the placenta. They thought that this indicated a disturbance of differentiation whereby more giant cells than normal were formed from the trophoblast. It is possible, however, that the total number of giant cells was not greater than in normal placentae of the same age, but that the effect was simply due to the cells being spread more thickly where there was a smaller surface to be covered.

As to the ultimate fate of the surviving placentae in rodents there is general agreement. Newton (1935) in the mouse, and Selye *et al.* (1935) and Kirsch (1938) in the rat, found, as we did, that abnormal placentae were delivered at full term together with the normal ones. The gradual separation of the placentae from the decidua basalis, which we observed, was evidently preparatory to their complete separation and expulsion at full term.

SUMMARY

1. Following experimental foetal death in the rat, the amnion and the inner wall of the yolk sac died and were resorbed, but the omphalopleure, allantois and placental trophoblast survived.

2. Growth of the surviving membranes was rapid up to the 16th day, but ceased after that.

3. Histological differentiation proceeded along normal lines except in the labyrinth where the foetal vessels were obliterated and their endothelium merged with the trophoblast to form a composite syncytium.

4. In the absence of the amniotic fluid the placenta was more rounded than normal and the giant-cell and spongy zones were thicker.

5. Two kinds of degenerative change were observed: (*a*) after destruction of foetuses on the 10th–12th days the peripheral parts of the placenta underwent haemorrhagic infarction in late pregnancy, (*b*) after destruction on the 16th–19th days there followed a gross oedema of the labyrinth.

6. At full term the abnormal placentae were expelled.

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Abbreviations used in the figures

AM = allantoic mesenchyme.
BC = blood clot.
BP = basal plate of trophoblast.
CT = cyto-trophoblast.
DB = decidua basalis.
DC = decidua capsularis.
DGC = disintegrating glycogen cells.
ES = entodermal sinus of Duval.
FBV = foetal blood vessel.
FVEN = foetal vascular endothelial nucleus.
GC = giant cells.
L = labyrinth.
MS = maternal blood sinus.
N = necrotic debris.
NDB = necrotic decidua basalis.
NDC = necrotic decidua capsularis.
NSZ = necrotic spongy zone.
O = omphalopleure.
OC = omphalopleuric cavity.
OL = oedematous labyrinth.
PT = placental trophoblast.
RMPE = Reichert's membrane and parietal entoderm.
SES = subepithelial stroma.
ST = syncytio-trophoblast.
SYS = splanchnopleuric wall of yolk sac.
SZ = spongy zone.
SZT = spongy zone trophoblast.
TN = trophoblastic nucleus.
UE = uterine epithelium.
UL = uterine lumen.
YSL = yolk sac lumen.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Transverse section normal uterus at the 10th day of pregnancy. Boundary between foetal and maternal tissues indicated by a white line. × 15.

Fig. 2. Transverse section uterus at the 12th day. Foetus destroyed at the 10th day. × 15.

Fig. 3. Transverse section uterus at the 13th day. Foetus destroyed at the 10th day (specimen *A*). × 15.

Fig. 4. Transverse section uterus at the 13th day. Foetus destroyed at the 10th day (specimen *B*). × 15.

Plate 2

Fig. 5. Transverse section uterus at the 15th day. Foetus destroyed at the 10th day. × 15.

Fig. 6. Transverse section uterus at the 19th day. Foetus destroyed at the 10th day. × 15.

Fig. 7. Normal uterus at the 10th day of pregnancy, showing decidua capsularis (*DC*), the bilaminar omphalopleure (*GC* and *RMPE*) and the splanchnopleuric wall of the yolk sac (*SYS*). (Part of Fig. 1.) × 98.

Fig. 8. Normal uterus at the 10th day showing the three zones of the placental trophoblast (*GC*, *SZ*, *BP*), the adjacent allantoic mesenchyme (*AM*) and the decidua basalis (*DB*). (Part of Fig. 1.) × 98.

Fig. 9. Part of normal labyrinth at the 12th day showing alternating foetal blood vessels (*FBV*) and maternal sinuses (*MS*) separated by trophoblastic syncytium (*ST*). × 196.

Plate 3

Fig. 10. Parts of the labyrinth and allantoic mesenchyme at the 12th day of pregnancy, after foetal destruction on the 10th day. (Part of Fig. 2.) × 196.

Fig. 11. Region adjacent to that shown in Fig. 10 to contrast the cellular (*CT*) and syncytial (*ST*) parts of the labyrinthine trophoblast. × 196.

Fig. 12. Part of anti-mesometrial uterine mucosa showing villous folds of the epithelium at the 13th day of pregnancy after foetal destruction on the 10th day. (Part of Fig. 4.) × 196.

Fig. 13. Part of labyrinth at the 14th day of pregnancy following foetal destruction on the 11th day, showing surviving allantoic mesenchyme (*AM*) closely invested by cytotrophoblast (*CT*). × 98.

Fig. 14. Part of placenta at the 14th day of pregnancy following foetal destruction on the 11th day. showing placental and omphalopleuric giant cells (*GC*) separated by a double layer of Reichert's membrane and parietal entodermal cells (*RMPE*). × 98.

Plate 4

Fig. 15. Part of normal labyrinth at the 15th day. × 392.

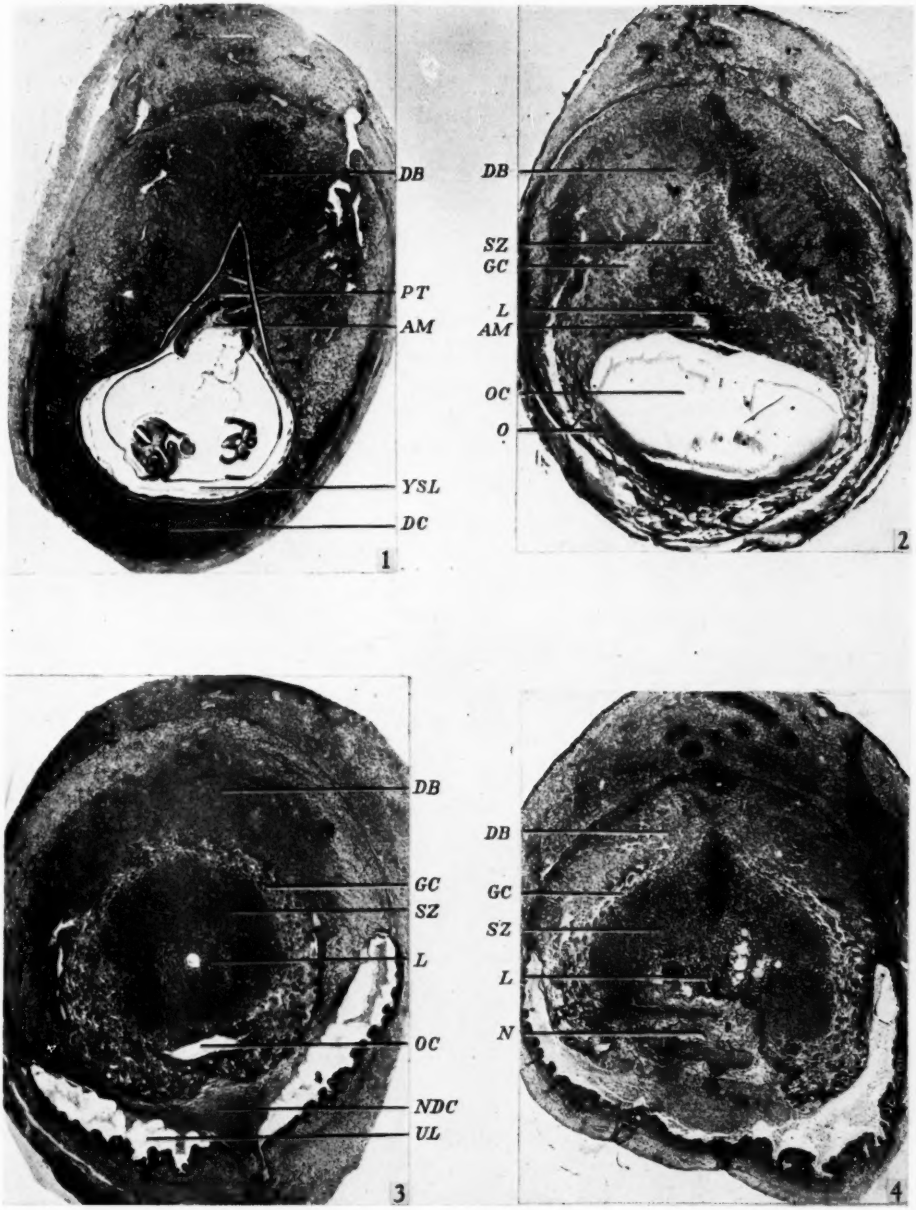
Fig. 16. Part of labyrinth at the 15th day following foetal destruction at the 10th day. × 392.

Fig. 17. Part of labyrinth at the 15th day following foetal destruction at the 12th day. × 392.

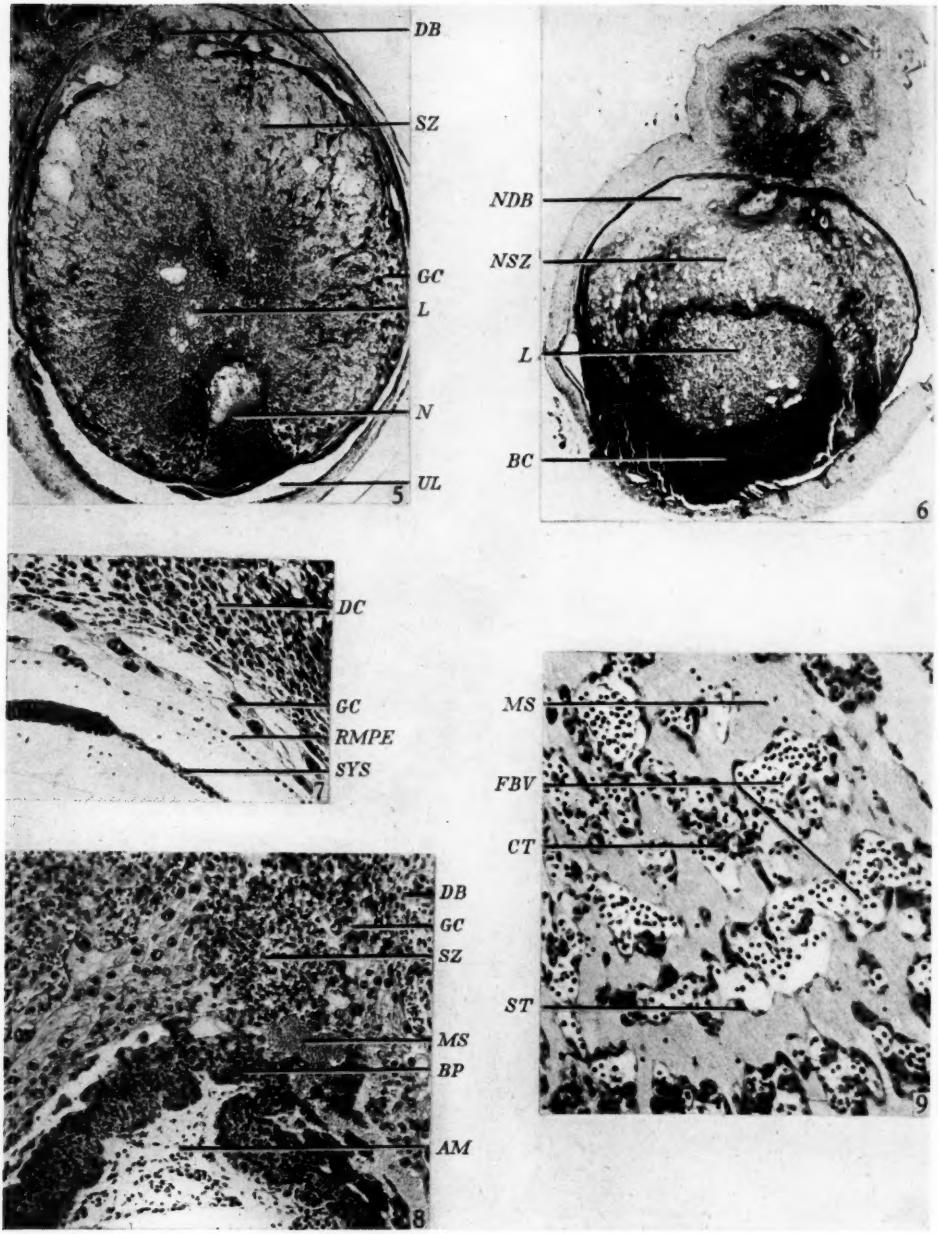
Fig. 18. Part of spongy zone at the 16th day following foetal destruction at the 10th day, showing disintegration of the glycogen cells. × 196.

Fig. 19. Part of labyrinth at the 21st day following foetal removal at the 16th day, showing oedematous swelling of the labyrinth (an area of minimal swelling chosen; cf. Fig. 20 at one-half the magnification). × 392.

Fig. 20. Part of labyrinth at the 21st day following foetal destruction on the 16th day, showing a surviving entodermal sinus of Duval in the midst of grossly oedematous trophoblast. × 196.



PRITCHARD AND HUGGETT—EXPERIMENTAL FOETAL DEATH IN THE RAT



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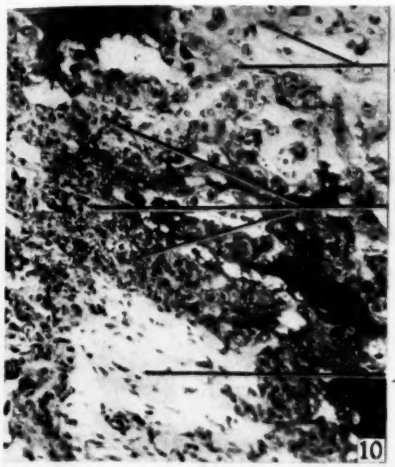

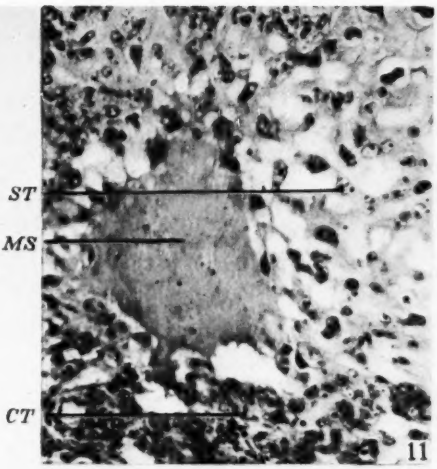

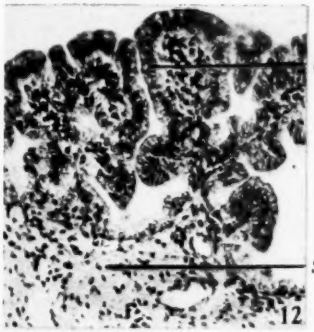

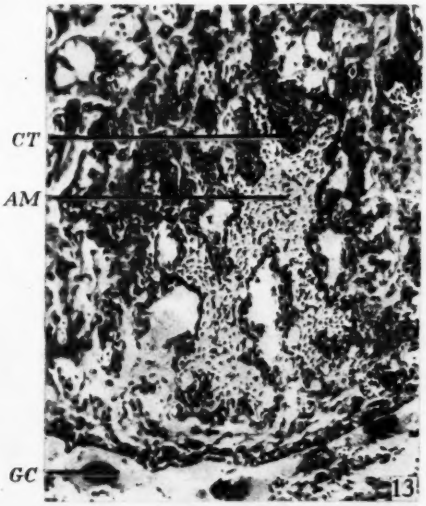

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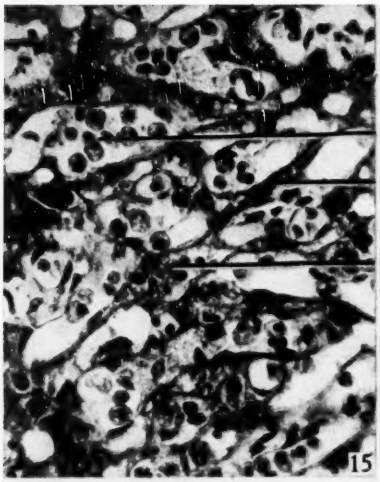
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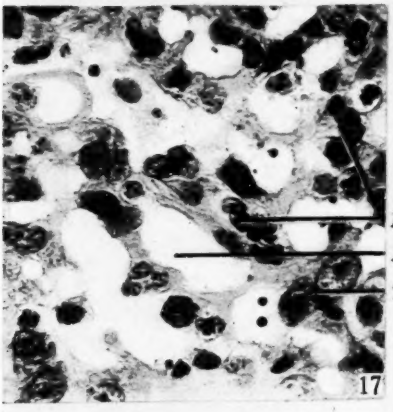
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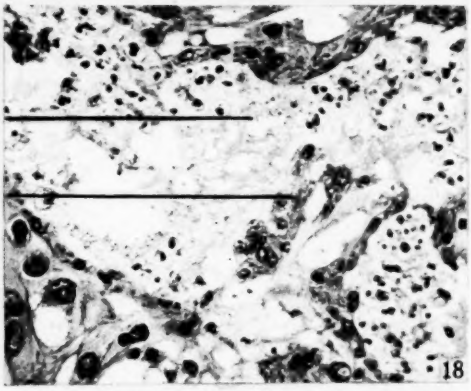
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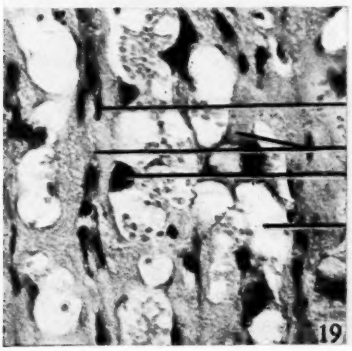
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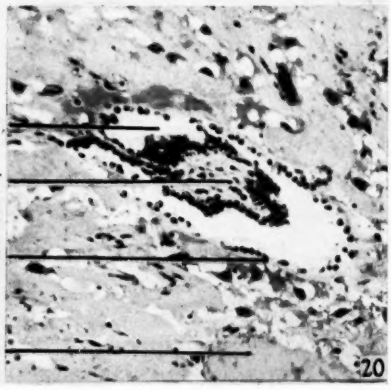
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ENDINGS PRODUCED BY SOMATIC NERVE FIBRES GROWING INTO THE ADRENAL GLAND

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New light has been shed on the mechanism of peripheral nerve regeneration by the experimental work of the past few years. It has been found that the re-establishment of a peripheral connexion has a profound influence on the diameter of regenerating fibres in the case of both sensory and motor somatic nerves. The nerve fibres which grow out from the central stump of an interrupted nerve are numerous and small. If these fibres are not allowed to reach their end-organs they fail to mature, so that the regenerated stretch of nerve contains numerous small fibres (Weiss, Edds & Cavanaugh, 1945; Sanders & Young, 1945, 1946). Those fibres which reach their end-organs increase to their normal diameter and in some way bring about the disappearance of the excess of small fibres.

However, this influence of the end-organ is not the only factor controlling the diameter reached by regenerating fibres. Young (1942) and Simpson & Young (1945) demonstrated that when a somatic nerve is united to the non-medullated anterior mesenteric nerve in rabbits medullated fibres are formed in the latter, though they are of smaller diameter than those produced by union of somatic to somatic nerve. A similar result was obtained following anastomosis of somatic nerve to the finely medullated greater splanchnic nerve. Hammond & Hinsey (1945) have shown similar effects with other nerves. These results show that the size reached by regenerating fibres depends on the diameter of the central stump fibres and on the size of the peripheral tubes into which they grow, as well as on their terminal connexions.

The present investigation deals with the distribution and nature of the nerve fibres which reach the adrenal following anastomosis of a somatic nerve as central stump to the greater splanchnic nerve. It is well established that in a normal animal preganglionic fibres run direct from the splanchnic nerves to end in the adrenal medulla (Elliott, 1913; Hollinshead, 1936; Young, 1939). The aim was to see whether the large somatic axons would grow to large size in the adrenal, and whether they would follow the pathways of the normal fibres to the gland, remaking the normal nerve plexus in the medulla. This constitutes a new test of the effect of central and peripheral influence on nerve regeneration. Should it prove that somatic fibres make large endings in autonomic plexuses this might provide a new method of investigating the more obscure points of peripheral autonomic nerve distribution, by using the readily identified somatic myelinated fibres as 'tracers'.

OPERATIVE METHOD

Adult rabbits were used. The abdomen was opened under nembutal and ether anaesthesia; the left greater splanchnic nerve was then identified and cut 1·5 cm. proximal to the anterior mesenteric ganglion, the central end being evulsed. The central ramus of one of the upper two lumbar nerves was then dissected free from the underlying muscle and cut 4 cm. from the lateral border of psoas. The proximal end was brought into apposition with the distal cut end of the greater splanchnic and joined by the application of a solution of human fibrinogen freshly made up from dried material and mixed with thrombin. The dried fibrinogen and thrombin were kindly supplied by the Lister Institute and proved very convenient for the purpose.

The animals were allowed to survive the operation for periods ranging from 100 to 200 days. Biopsy was then carried out under nembutal anaesthesia, both adrenals, and the site of anastomosis, being removed for fixation.

HISTOLOGICAL METHODS

Normal glands were stained by the Bielschowsky-Gros, Cajal, and Bodian methods, but the latter only was used in staining the experimental glands. Certain modifications have been introduced into the Bodian method and the exact technique used was as follows:

(1) Fixation of the glands in Carnoy's solution for 2 hr. (absolute alcohol 60 parts, chloroform 1 part, glacial acetic acid 1 part).

(2) Dehydration and paraffin embedding.

(3) Sections were cut at 15μ thickness and stained with 1% Protargol in distilled water (Protargol supplied by Winthrop Chemical Co., New York). Sections were left in this solution for 24 hr. at 37° C.

(4) Distilled water—10 min.

(5) 1% oxalic acid for periods varying from 10 sec. to 1 min.

(6) Reduction. A solution of hydroquinone 1%, sodium sulphite 5% was used. Reduction is complete in about 1½ min.

(7) Wash in running tap water for 1 min. followed by distilled water for 1 min.

(8) Toning, 0·1% gold chloride 4 min.

(9) Oxalic acid 2% for 2–3 min. This stage was observed under the microscope and the process stopped when the fibres appeared black on a grey background.

(10) Wash in distilled water for 1 min.

(11) Fix in hypo, wash, dehydrate and mount in balsam.

If the sections appear purple after stage 9 the period in oxalic acid in stage 5 should be increased.

NORMAL INNERVATION OF THE ADRENAL

To obtain a standard of comparison the innervation of the normal adrenal gland was reinvestigated using Bielschowsky, Cajal and a modified Bodian method. The results confirmed the work of Hollinshead (1936), Willard (1936) and Young (1939). The subcapsular plexus gives off a number of nerve bundles which pass through the cortex towards the medulla in a radial direction. These bundles occasionally give off branches while passing through the cortex, but in spite of careful search no nerve endings were found in the cortex adjacent to the nerve bundles, so presumably the few branches found in this region eventually reach the medulla. Alpert (1931) and others have claimed that nerve fibres end around cortical cells, but it is now generally held that this tissue has no direct innervation, all nerves within it passing either to blood vessels or to the medulla. At the cortico-medullary junction the nerve bundles can often be seen to bend, becoming oriented parallel with the margin of the medulla before they finally enter it.

In marked contrast to the poverty of fibres in the cortex, the medulla is very richly innervated. Nerve bundles can be traced inwards from the trunks at the cortico-medullary junction; usually they accompany the blood vessels. They soon branch profusely to form a nerve plexus around the lobules of chromophil tissue cells (Pl. 1, fig. 1). From this plexus fine nerve fibres are given off which ramify among the cells of the lobule, thus ensuring that all the chromophil cells are in contact with at least one nerve fibre.

It is probable that stimulation of the medullary cells is effected by direct contact with the fine fibres of the nerve network. Bulbs of various sizes have been described (Hollinshead, Willard), some situated in the course of the fibre and some at its termination, but they are not sufficiently numerous to be the main factor in the transmission of impulses. Thus Willard (1936) found that there were only 1·4 boutons per fifty chromophil cells in adult guinea-pigs.

No nerve cells were found in the medulla of the rabbit, although several preparations show well-stained ganglia in the extracapsular tissue.

EXPERIMENTAL FINDINGS

(1) *In the cortex*

In the glands innervated by somatic nerve fibres marked abnormalities are very clearly seen. First there are fibres far thicker than any normally present, derived from the axons of the donor nerve. A proportion of these pass radially in the nerve trunks, as in the normal arrangement, through the cortex to the medulla. In the cortex considerable lengths of thick fibre can be seen in even a single section (Pl. 2, fig. 5). Very many of the new fibres, however, have escaped from the nerve trunks into the neighbouring cortex, where they wander for some distance from the parent trunk (Pl. 2, figs. 4, 5). They are thus found in groups scattered throughout the cortex, each group having in its centre

a nerve trunk (Pl. 2, fig. 4). An example of a fibre actually leaving a trunk is shown in Pl. 2, fig. 5.

Only short lengths of these escaped fibres were seen in any one section, and in general they tended to be disposed tangentially, which facts indicate a tortuous course. Indeed, it proved very difficult to trace them in continuity even in serial sections.

It must be stressed again that the fibres found in the cortex adjacent to the nerve trunks in a normal gland are few in number and remain close to the parent trunk. These thick fibres in the hetero-innervated glands were present in far greater number and had wandered much farther from the trunk than is the case in the normal arrangement (Pl. 2, fig. 4). It is clear therefore that these fibres had not grown down pre-existing sheaths but had escaped from their tubes in the trunk.

These thick fibres showed an irregular undulating or varicose outline in their whole course, with frequent branching. The great majority did not reach the medulla but ended in the cortex. Their endings had the form of a club-shaped expansion with numerous short bulbous outgrowths, giving an irregular coarse stellate appearance (Pl. 2, fig. 7). Here and there a much finer filament was seen springing either from the main club-shaped expansion or from one of its excrescences (Pl. 2, figs. 6, 7). These filaments ran for a short distance amongst the cortical cells and terminated in a fine or coarse bouton type of ending.

In addition to these thick fibres, fine fibres, regular in outline and histologically similar to those found in the medulla of a normal gland, were seen in large numbers around the nerve trunks, and spread out for a short distance into the surrounding cortex (Pl. 2, fig. 5). In a few cases these fine filaments could be seen to arise from one of the thick nerve fibres in the trunk but the majority appeared to have no relation to them. Furthermore, they differed from the thick fibres in their mode of distribution, they ran tangentially and formed a plexus amongst the cortical cells. The result was a most striking increase in the overall richness of nerve fibres in the cortex. However, it must be emphasized that these nerve fibres were all close to the bundles running through the cortex, large areas of cortical tissue situated at a distance from such bundles were quite devoid of nerve fibres, as in the normal gland.

(2) *Pattern in the hetero-innervated medulla*

Here again large and small fibres were present. They entered the medulla as components either of a nerve bundle or of the cortical plexuses. In contrast to the appearance in the normal medulla, where the intercellular plexus is found developed in a uniformly rich manner in all areas (Pl. 1, fig. 1), it is not so developed in the medulla of the experimental gland, especially in those surviving 100 days (see below). A network similar to the normal was found only in discrete areas comprising a small portion of the whole, in most cases

less than one-third. These scattered, richly innervated areas presumably contain fibres derived from the lesser splanchnic and lumbar sympathetic chain and not interrupted by the operation. So the areas of medulla showing a relative poverty in their fine fibre innervation (and comprising at least two-thirds of the whole) can be regarded as those to which the lesser splanchnic and lumbar sympathetic make no contribution (see Young, 1939). Whereas at 100 days the poverty of the medullary plexus in these areas was striking, in one rabbit killed at 200 days there was a definite advance towards the normal density of innervation.

The thick fibres in the medulla occurred singly or in small groups, in the form of either straight or curved lengths, showing a slightly undulating outline and branching infrequently (Pl. 1, figs. 2, 3). These fibres were nowhere seen to develop a plexus arrangement. They appeared to end abruptly with no more than slightly bulbous expansion at their tips. They did not display any of the elaborate behaviour seen in the cortex. The fine fibres were found in small numbers amongst the chromophil cells. They branched frequently, but after 100 days the striking feature was that these fibres had not reproduced the normal rich medullary plexus. Even at 200 days there were considerable areas of medulla still poorly innervated.

DISCUSSION

Behaviour in the cortex

It is clear that the innervation pattern described above has been produced by the interaction of at least two abnormal factors. In the first place the regenerating somatic fibres often escape from the sheaths to invade the cortical tissue. Secondly these escaped fibres were subjected to grossly abnormal conditions; the adrenal cortex is densely cellular and produces a considerable mechanical obstruction to growth.

Many observations have been carried out to determine the effect of obstruction on the growing axon. Cajal (1928) investigated the effect of ligatures tied round regenerating nerve trunks and describes how the fibres proximal to the constriction formed large balls, with very fine exploratory filaments sent out from them in an attempt to force a path or find an alternative route. Speidel (1933), while observing the growth cones of regenerating nerves *in vivo*, described the formation of giant cones when formidable obstructions were reached. It has also been observed that during the early stages of normal regeneration, even in the absence of any artificial obstruction, the central end of the axon attached to the cell often swells. This expansion of the axon at the tip of the central stump is then a normal phenomenon in regeneration, but it is greatly exaggerated where there is obstruction (Weiss *et al.* 1945). This has led to the view that the nerve cell exerts a turgor pressure on the whole unit. The fine filaments arising from the club-shaped masses presumably represent an outflow into such small cracks as are available.

The thick fibres

The most likely explanation of the large club-shaped masses of axoplasm found in the cortex of the experimental animals is, then, that they are the result of obstruction to the longitudinal growth of the thick somatic axons at these points. After an axon became held up by the cortical cells the turgor pressure from the nerve cell continued to pour axoplasm into the tip.

The fine fibres

There are two possible sources of these fibres: (*a*) they may be derived from the autonomic and pain fibres in the somatic nerve, (*b*) some are definitely branches of the thick fibres. One example was found of a fine plexus being formed in the cortex from the fibre taking origin from a thick axon. It must be stressed, however, that most of the fine filaments arising from the thick fibres terminated in boutons a short distance from their source. It is probable that the fine fibres were derived to some extent from both these sources.

Behaviour in the medulla

The adrenal medulla consists of groups of chromophil cells surrounded by the sinusoidal venules. It is a far less compact tissue than is the cortex and hence obstruction phenomena would be expected to be less. As a result the thick fibres do not produce the club-shaped terminations found in the cortex, but rather form straight or curved rods with only a slight swelling at the tip.

Another striking feature is the absence of any formation of a plexus by these thick fibres. It would be interesting to investigate whether any adrenalin is produced when these somatic nerves are stimulated. Theoretically adrenalin could be produced, for both the somatic fibres and the preganglionic sympathetic fibres which normally innervate the adrenal medulla secrete acetylcholine (acetylcholine production was demonstrated in the adrenal by Feldberg, Minz & Tsudzimura (1934). Experiments to investigate this possibility are now being undertaken.

The fact that somatic fibres growing into the adrenal form fibres which are much larger than those normally present confirms the finding of Simpson & Young (1945) and Hammond & Hinsey (1945) that the size of a parent fibre has an influence on that of fibres regenerated from it, irrespective of their termination.

CONCLUSION

The conclusions so far are, then, that the influence of a central somatic fibre persists in a foreign autonomic tissue and determines that the regenerating axons shall be thick, but that this influence is not sufficiently strong to produce the formation of somatic end organs. It can also be concluded that the adrenal is unable to impose its own nerve pattern on the ingrowing fibres.

We have seen that nerve regeneration is not purely a non-specific process of downgrowth at random, but is markedly influenced by both central and

peripheral factors. It is only when an axon grows down to its own periphery and when the periphery is innervated by its own central axon that the normal pattern is reproduced. The part that function plays in the regeneration process is at present little understood, it may be that when an axon reaches a suitable periphery it begins to function (at first inefficiently) and that this acts as a stimulus which brings about the modelling of the terminal ramifications necessary for the more efficient carrying on of these functions. Further experiments are necessary to elucidate the part function plays in nerve regeneration.

SUMMARY

1. Somatic nerve fibres made to grow into the great splanchnic nerve and adrenal gland of rabbits produced the following abnormalities: (*a*) Many fibres escaped from the nerve bundles running through the cortex and proceeded for some distance among the cortical cells. They end as large bulbs, presumably when obstructed. (*b*) Fibres reaching the medulla end as large rods and do not form typical plexuses.

2. It is therefore demonstrated that the type of nerve termination produced in this gland during regeneration is affected by the nature of the central stump from which the fibre proceeds.

I wish to thank Prof. J. Z. Young for his constant criticism and advice and for reading the manuscript.

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EXPLANATION OF PLATES

All figures are of adrenal glands of rabbits stained with Bodian's method.

Plate 1

Fig. 1. Normal medulla. The rich plexus is found amongst the chromophil cells.

Fig. 2. Hetero-innervated medulla, 100 days. The thick and fine fibres are seen running together inside the nerve bundle.

Fig. 3. Cortico-medullary junction of hetero-innervated gland, 100 days. A thick fibre is seen leaving a bundle to branch in the surrounding tissue. *b*, bundle cut transversely.

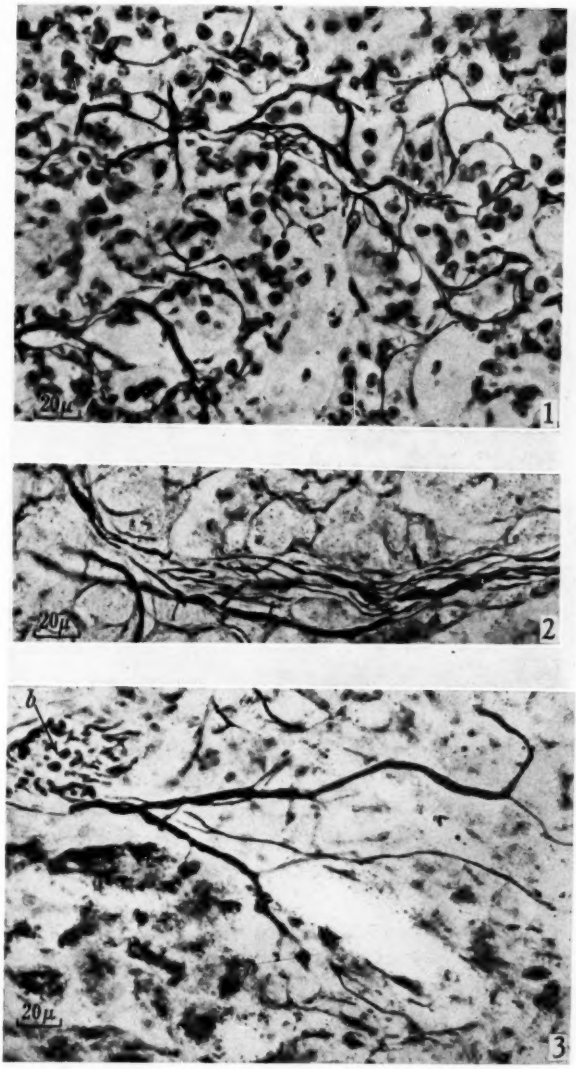
Plate 2

Fig. 4. Hetero-innervated cortex, 100 days. A nerve bundle (*b*) is cut transversely. A number of thick somatic fibres which have escaped into the neighbouring cortex are seen.

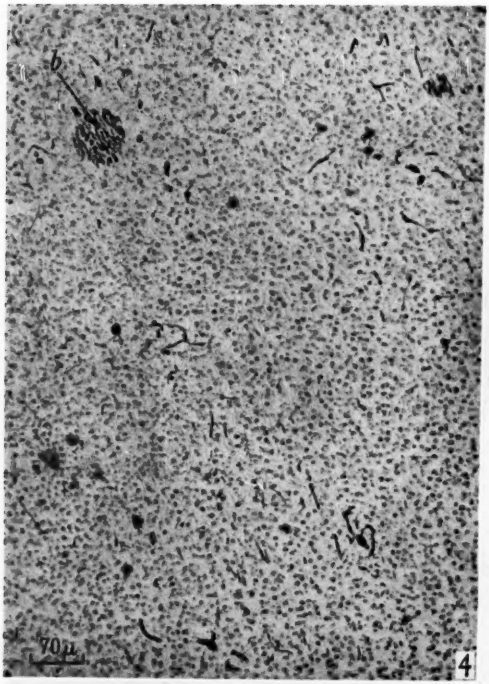
Fig. 5. Hetero-innervated cortex, 100 days. A nerve bundle is seen passing through the cortex. Most of the fibres are fine, but a thick one can be seen escaping into the cortex. Numerous fine fibres are also seen running between the cell columns.

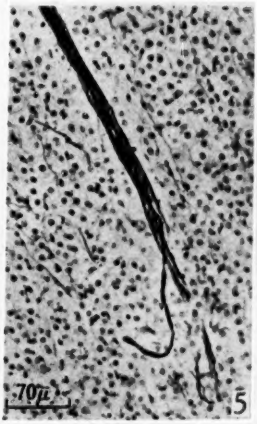
Fig. 6. Hetero-innervated cortex, 100 days. A higher power view of the cortex around a nerve bundle (*b*). The varicose appearance of the thick fibres can be seen.

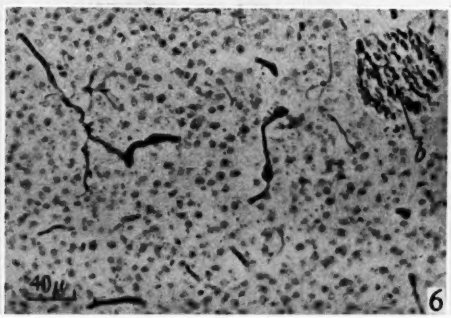
Fig. 7. Hetero-innervated cortex, 100, days showing the club-shaped terminations of the somatic fibres. Fine processes (*p*) ending in boutons can be seen arising from these masses.

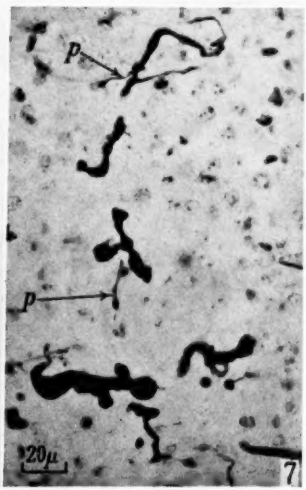


Figs. 1—3









Figs. 4—7

HEADFORM AND HUMAN EVOLUTION

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INTRODUCTION

The progressive rise in mean cephalic index, which is such a striking feature of human evolution, has lately attracted much attention, particularly from Kappers (1935*a*, *b*, 1936*a*, *b*, 1937, 1939, 1945) and Weidenreich (1940, 1945). Kappers considers the various aspects of the problem only as they affect man. Weidenreich, on the other hand, adopts a broad biological approach which adds very materially to our knowledge from all points of view. However, his conclusion (1945, p. 50) that 'Brachycephalization, therefore, means a better adjustment of the skull to the erect posture by adopting a more complete globular form' carries little conviction for there is no evidence that the most brachycephalic peoples are better adjusted to the upright posture than are the most dolichocephalic. Weidenreich's belief that increasing brachycephalization is associated with human evolution generally is certainly correct, but some other explanation should be sought for the coincidence.

Ever since its inception the cephalic index has remained the major criterion of human affinities and development. On the other hand, anyone who has measured a series of skulls must have realized that the loci from which the index is calculated vary from skull to skull, and that the index expresses something different for every skull. Nevertheless, Morant (1928, p. 355) claims that 'only one character has been found which is capable of making an absolute distinction between more than one of the groups [i.e. of humans] and all the others: that is the cephalic index'. Thus the value of the index seems to be well established, but it can be accepted only as a total response to a number of variable factors, some of which are probably still quite obscure.

This is the attitude adopted in the present inquiry which seeks some unifying principle under the apparent diversity of a multitude of observations. The problem of headform is considered in its relation to evolution, race, size of jaws, heredity and environment, internal form and capacity, stature, sex and individual development. The fact that such a unifying principle does seem to emerge justifies this addition to the already extensive literature on the subject.

MATERIALS

Data relating to the Australian aborigine are employed as a control on a number of the various factors considered here. The cranial data comprise measurements on both adult and juvenile skulls in this Department and, especially, in the Adelaide Museum. For adults, fifty male and fifty female skulls were measured: they represent about 10 % of the total material available, are representative of all parts of Australia and form a strictly random sample since they were taken just as they came. The fact that the mean cranial

Table 1. *Length, breadth, cranial index, facial angle and alveolar index of fifty male and fifty female Australian aboriginal skulls*

Male					Female				
Length (mm.)	Breadth (mm.)	Cranial index	Facial angle	Alveolar index	Length (mm.)	Breadth (mm.)	Cranial index	Facial angle	Alveolar index
187	120	64·2	74	102·9	170	113	66·5	81	100·0
199	130	65·3	76	102·8	189	126	66·7	79	100·0
192	127	66·2	77	97·1	186	125	67·2	86	100·0
186	124	66·7	80	101·9	183	123	67·2	80	109·9
193	129	66·8	78	106·8	174	117	67·2	75	107·7
197	132	67·0	76	105·8	172	117	68·0	77	109·6
188	126	67·0	77	97·0	180	123	68·3	84	100·0
192	129	67·2	75	104·7	174	119	68·4	77	105·3
190	128	67·4	78	102·0	181	124	68·5	75	108·2
184	124	67·4	80	95·2	174	120	69·0	78	106·5
195	132	67·7	78	103·0	172	119	69·2	80	103·3
202	138	68·3	84	101·8	185	128	69·2	74	107·2
187	128	68·5	76	103·1	185	128	69·2	77	106·4
173	119	68·8	76	103·1	176	122	69·3	75	100·0
176	121	68·8	76	103·9	179	124	69·3	77	102·1
184	127	69·0	81	100·0	179	124	69·3	83	100·0
187	129	69·0	75	105·3	173	120	69·4	78	104·1
190	131	69·0	80	103·1	178	124	69·7	78	105·2
192	133	69·3	75	105·0	172	120	69·8	76	109·4
192	133	69·3	87	96·9	173	122	70·5	79	102·2
183	127	69·4	84	98·0	181	128	70·7	80	105·5
190	132	69·5	76	101·0	178	126	70·8	80	104·5
188	131	69·7	78	110·0	170	121	71·2	76	101·1
182	127	69·8	80	98·2	184	131	71·2	80	102·1
182	128	70·3	81	97·9	182	130	71·4	70	107·5
185	130	70·3	73	101·9	186	133	71·5	87	95·0
190	134	70·5	73	108·6	172	123	71·5	77	103·3
194	137	70·6	76	109·5	176	126	71·6	75	108·3
187	132	70·6	75	108·1	176	127	72·2	76	106·3
187	132	70·6	84	100·0	180	130	72·2	76	106·3
193	137	71·0	85	96·1	177	128	72·3	73	113·7
196	140	71·4	80	100·0	184	133	72·3	72	106·5
182	130	71·4	83	97·9	167	121	72·5	82	105·6
183	131	71·6	82	100·0	171	124	72·5	77	105·3
195	140	71·8	75	110·2	172	125	72·7	81	101·1
185	133	71·9	77	103·8	181	132	72·9	78	104·2
182	131	72·0	81	100·0	176	129	73·3	79	100·0
183	132	72·1	73	106·9	174	128	73·6	81	95·7
174	126	72·4	75	103·9	182	134	73·6	73	103·2
175	127	72·6	78	100·0	167	123	73·7	79	103·2
186	135	72·6	80	100·0	183	135	73·8	76	110·6
188	137	72·9	78	102·0	176	130	73·9	81	98·9
187	137	73·3	79	104·0	188	139	73·9	79	100·0
187	138	73·8	83	100·0	166	123	74·1	80	100·0
180	133	73·9	78	105·2	169	126	74·6	80	100·0
176	130	73·9	82	100·0	168	126	75·0	76	101·1
189	140	74·1	78	100·0	165	124	75·2	78	103·2
176	131	74·4	73	105·4	169	127	75·2	76	104·4
193	144	74·6	83	105·3	168	128	76·2	75	103·2
190	143	75·3	81	100·0	162	126	77·8	79	104·2
Means 187·080	131·300	70·224	78·460	102·306	176·100	125·480	71·306	78·020	103·822
Standard deviation 6·521	5·489	2·631	3·489	3·700	6·582	5·015	2·678	3·310	3·827
Corr. coeff. L./B. 0·530 ($P<0·001$)					0·543 ($P<0·001$)				
Corr. coeff. F.A./Alv. I.			−0·620 ($P<0·001$)					−0·619 ($P<0·001$)	
Corr. coeff. L./F.A. 0·096 (n.s.)					0·016 (n.s.)				
Corr. coeff. L./Alv. I. 0·153 (n.s.)					0·046 (n.s.)				

indices (male: 70·2; female: 71·3) conform closely to figures given by Turner (1884), Hrdlička (1928) and the pooled data of Martin (1928), given here in Table 6, supports the belief that the sample is representative. Herskovits (1926) believes that the correlation between head length and head breadth is an index of group homogeneity; the highest coefficient he gives is 0·586 for Teneriffian males and for Batatelu. If his view is correct this group of aborigines is exceptionally homogeneous, having a length-breadth correlation coefficient of 0·530 for the male and 0·543 for the female, with the probability of less than 1 in 1000 that the result is due to chance. These data are recorded in Table 1. Throughout this paper the customary level of significance has been adopted, namely, that for a statistical index to be real—i.e. unlikely to have occurred merely as the result of chance—the probability of its occurring by chance must be less than five times in a hundred trials ($P < 0{\cdot}05$).

The juvenile material comprised forty-two skulls, ranging from newly born to late adolescent. The ages were estimated according to Campbell's (1925) observations. The data are presented in Table 2.

Information relating to the stature and cephalic index of fifty living male and fifty living female aborigines is compiled from the work of Burston (1913), Campbell & Lewis (1926) and Campbell & Hackett (1927). They represent respectively groups from the Northern Territory, South Australia and Central Australia. The figures were taken strictly as they came: all the female figures from Campbell and Lewis and from Campbell and Hackett were recorded with an equal number of the males; the totals were completed by adding to each an equal number of males and females from Burston. The results are given in Table 3.

The ectocranial and endocranial indices comprise the measurements of twenty-one skulls given by Weidenreich (1945, Table 1) plus the corresponding figures for twenty others from the collection in this Department. The data, and the provenance of the skulls, appear in Table 4.

For comparison with the aboriginal data measurements relating to stature and cephalic index (Table 5) and cranial index (Table 6) were collected from a number of sources, but chiefly from Martin (1928).

Finally, to obtain a figure for the mean cephalic index of the newly born European the heads of forty infants were measured. One female proved to be of part-aboriginal origin and was omitted from the series. The remainder comprised sixteen males and twenty-three females; their ages ranged from 1 to 22 days and none gave any evidence of persistent birth moulding.

FACTORS WHICH MAY BE CONCERNED IN THE DETERMINATION OF HEADFORM

(1) *Evolution*

The progressive rise in the mean human cranial index from early man up to the present is too well known to require more than brief attention; moreover, the evidence is fully surveyed by Weidenreich (1945). Outstanding features of

the rise are that it seems to have been practically universal, and everywhere approximately contemporaneous. Another important point is that while the mean index started low there have always been individuals, or even small

Table 2. *Cranial index, facial angle and alveolar index of forty-two Australian aboriginal juvenile skulls*

Dental epoch	Estimated age	Cranial index	Facial angle	Alveolar index	Remarks	Mean cranial index
Prior to eruption of first permanent molar	Term	80·0 +	83	100·0	Birth moulding	80·0 +
	18 months	72·8	80	—	No basion	74·8 (2)
	18 months	76·7	91	100·0	—	
	2–5 years	75·3	90	94·7	Basion damaged	73·2 (4)
	2–5 years	78·0	82	100·0	—	
	2–5 years	72·0	89	—	No basion	
	2–5 years	67·4	83	—	No basion	
	5 years	74·1	79	101·2	—	73·0 (2)
	5 years	71·8	83	—	No basion	
Prior to eruption of second permanent molar	6 years	80·4	83	102·6	Prosth. damaged	73·5 (4)
	6 years	75·1	84	102·3	—	
	6 years	71·1	86	—	No basion	
	6 years	67·4	80	—	No basion	
	7 years	71·4	83	102·4	—	73·1 (2)
	7 years	74·8	78	102·4	—	
	8–10 years	73·5	—	—	No base	72·1 (8)
	8–10 years	78·1	81	96·6	—	
	8–10 years	69·9	77	102·9	—	
	8–10 years	70·5	80	96·8	—	
	8–10 years	63·6	85	96·7	—	
	8–10 years	69·6	80	102·4	—	
	8–10 years	77·9	82	100·0	—	
	8–10 years	73·3	82	97·8	—	
	11 years	72·2	84	95·5	—	72·4 (8)
	11 years	73·6	84	96·7	—	
	11 years	75·7	79	102·2	—	
	11 years	69·9	85	99·0	—	
	11 years	73·6	80	100·0	Known female	
	11 years	71·1	82	105·7	—	
	11 years	70·6	84	101·1	—	
	11 years	72·2	82	100·0	—	
Prior to eruption of third permanent molar	12–15 years	75·3	81	96·6	Arbitrary sub-division based on skull length under 171 mm.	74·4 (6)
	12–15 years	71·8	78	106·4		
	12–15 years	77·1	80	101·1		
	12–15 years	76·3	84	98·9		
	12–15 years	73·5	80	103·4		
	12–15 years	72·4	77	100·0		
	16–20 years	71·1	84	103·2	Arbitrary sub-division based on skull length 171 mm. or over	71·9 (5)
	16–20 years	72·0	79	101·1		
	16–20 years	72·0	76	106·8		
	16–20 years	70·1	83	104·4		
	16–20 years	74·2	82	100·0		
Means for 5-year periods	Birth	80·0 + (1)	83 (1)	100·0 (1)	Figures in brackets represent the number of observations upon which the means are based	
	1–5 years	74·0 (8)	84·4 (8)	99·2 (4)		
	6–10 years	72·6 (14)	81·6 (13)	100·3 (11)		
	11–15 years	73·2 (14)	81·4 (14)	100·7 (14)		
	16–20 years	71·9 (5)	80·8 (5)	103·1 (5)		

groups, with an index well above the mean (Weidenreich); subsequent elevation of the mean seems to have been due rather to expansion of such groups *in situ* than to the introduction of a new factor.

(2) *Race*

The term 'race' is objectionable, both on politico-sociological grounds and because it is used equally for the whole human species and for sections of that species. It is hard to find an adequate substitute, but wherever practicable the term 'group' is employed here instead.

The fact that isolated groups may by inbreeding attain stable and characteristic forms of head (compare, e.g. the Armenian with the Australian aborigine) is undoubtedly the basis for the belief that headform is an indication of affinity. Thence arose the view that the progressive brachycephalization in Europe betrays progressive admixture with brachycephalic Asiatics. This so-called 'substitution theory' is denied by Hug (1940) and by Weidenreich (1945). Weidenreich points out that there is no evidence that the supposed home of this brachycephaly actually harboured brachycephals at the alleged time of invasion, nor is there any evidence of contemporaneous brachycephalization in the regions over which the invaders must have passed. Moreover, the presumptive descendants of the Asiatics can have inherited only their rounder heads for there is no trace of Asiatic influence in the facial skeleton. Gerhardt (1938) denies that the cephalic index has any racial significance: he believes that brachycephaly is due to a number of factors and has often appeared independently, probably by mutation. Kappers, too, seems to favour the idea of mutation. At all events, it must be agreed that there is little justification for attributing the increasing brachycephalization in Europe, at least, to Asiatic infiltration.

(3) *Relation to jaw development*

Since the dolichocephaly of early man was usually associated with marked prognathism, and since the evolutionary record shows a progressive diminution in both features (e.g. Morant, 1935) and since, further, the Australian aborigine exhibits definite increase in both during his ontogeny (Table 2, Fig. 1), the possibility that skull length and jaw length are related cannot be ignored. It is conceivable, indeed, that the longer skull serves as a mechanical balance for the heavier jaws. In pursuing this matter it may be borne in mind that even in the dolichocephalic and prognathous negro the part of the head behind the occipital condyles weighs 18 % less than that in front of the condyles (Schultz, 1940).

The possibility of some such relationship was checked in the Australian skulls, both adult and juvenile. The official measure of prognathism is the 'facial angle'—the angle the nasion-prosthion line makes with the Frankfurt horizontal. However, this angle is a measure of alveolar prognathism (Parsons, 1930) or prodenty (Weidenreich, 1945), rather than of true facial prognathism so, as a check, the 'alveolar index' of Flower (basion-prosthion/basion-nasion) was also determined wherever possible. There is (Table 1) fair correlation between the two expressions (male $-0{\cdot}620$; female $-0{\cdot}619$), with a probability of less than 1 in 1000 that this is due to chance.

Any relationship between head length and prognathism should give a good correlation with either facial angle or alveolar index. In fact (Table 1), there is no significant correlation in either case or in either sex. In the adult female, indeed, although the mean cranial index is higher than in the male, prognathism as measured by either criterion is greater (Table 1, Fig. 1). The difference in facial angle is not significant, but the difference in alveolar index is, since the probability that it is due to chance is less than 1 in 20. Greater female prognathism occurs in other groups (Martin, 1928) and, as it has some significance, must be a secondary sexual character. In the present context,

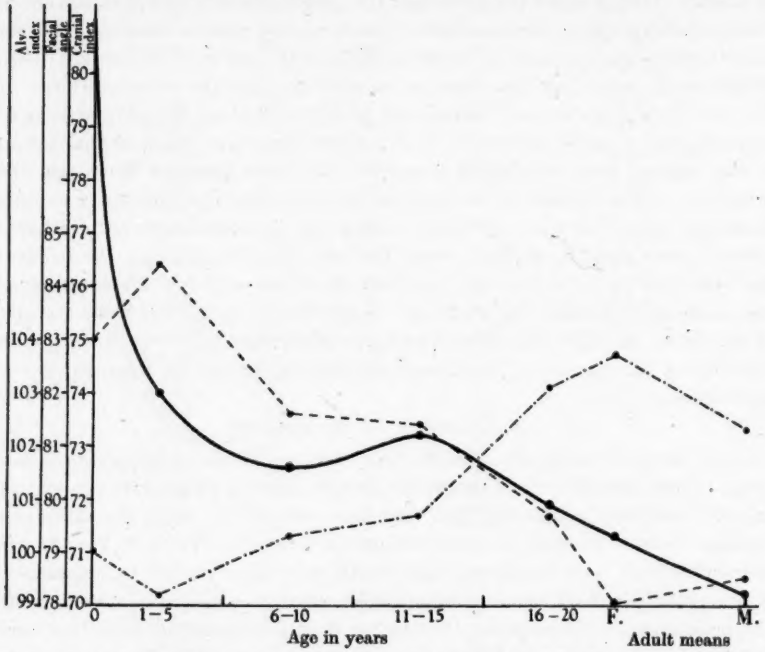

Fig. 1. Developmental changes in cranial index (smoothed-out curve), facial angle (broken line) and alveolar index (dots and dashes). Data: Tables 1 and 2.

this character strengthens the evidence of the correlation coefficients that there is no relationship between head length and jaw length. These two features must, then, vary independently.

Such independent variation is in accord with the more general observations of Davenport (1926), Huxley (1932), Burkitt (1946) and others, but is contrary to Weidenreich's (1940) belief (p. 435) that 'Phylogenetic enlargement of the brain alone is sufficient to transform "automatically" the entire skull with all its peculiarities.' Further evidence of independent variation in the skull—some of it collected by Weidenreich himself—will be given later. In the mean-

time it may be noted that the series of dogs upon which Weidenreich's views are based is far from representative. Certainly, the bulldog, King Charles's spaniel and pekinese have both round heads and short jaws, but many others in the 'dwarf' series—dachshund, miniature schnauzer, Scottish terrier, wire-haired fox terrier, cairn, sealyham, etc.—which have short heads have decidedly long jaws. Short-headedness is correlated with dwarfism but it is not necessarily associated with shortening of the jaws.

(4) *Heredity*

The fact that isolated inbreeding can produce a fairly stable and constant mean cephalic index, and the further fact that interbreeding between diverse stable groups introduces modifications in the descendants, indicate that hereditary transmission is a factor to be reckoned with. However, the inheritance of cranial characters seems to be a complex affair (Fawcett & Pearson, 1898; Frets, quoted by Kappers, 1945 and Weidenreich, 1945; and others), probably because so many independent variables are involved in the process that it cannot readily be expressed in simple Mendelian factors. In the case of the cephalic index, Weidenreich (1945) states that head length and head breadth vary independently, a view supported by the observations of Dunn (1928) on the cranial characters of pure and mixed Hawaiians and by those of Spier (1929) on native- and American-born Japanese. Further evidence of the complexity of this problem emerges from the measurements made by Sullivan (1919) on the 'Samar' united twins of whom Lucio had a cephalic index of 80·8, Simplicio an index of 85·5.

However, while the genetics of headform are not simply resolvable, it must be conceded that within a homogeneous group under stable conditions the cephalic index remains constant for a long time, and this must be due mainly to hereditary transmission.

(5) *Environment*

Boas (quoted from Martin, 1928; Kappers, 1935*b*; and Boas, 1940) was probably the first to adduce substantial evidence in support of the view that change in environment may influence the headform of the new generation. The suggestion has been attacked, notably by Pearson & Tippett (1924), but has received so much independent support (see Guthe, 1918; Hirsch, 1927; Klein, 1935; Kappers, 1935*b*, 1945) that it must be taken seriously into account.

To summarize briefly: the observations indicate that those with a high mean native cephalic index tend to show a fall in the first American-born generation; those with a low index tend to show a rise; intermediate groups betray little change. The best substantiated example of a significant fall is in Jews—from a native cephalic index of 83 to an American-born one of 81·4; an example of a rise is in Sicilians—from a native mean of 77·7 to an American-born mean of 81·5. Swedes, Scots and Italians, with native indices near 80 vary little, if at all. Similar observations are recorded by Klein (1935) on Askenasim

Table 3. *Stature, head length and breadth, and cephalic index of fifty male and fifty female Australian aborigines. Data: Burston* (1913), *Campbell & Lewis* (1926), *Campbell & Hackett* (1927)

	Male				Female			
	Stature (mm.)	Head length (mm.)	Head breadth (mm.)	Cephalic index	Stature (mm.)	Head length (mm.)	Head breadth (mm.)	Cephalic index
	1538	187	142	75·9	1426	181	134	74·0
	1555	181	142	78·5	1459	179	142	79·3
	1567	185	140	75·7	1463	193	133	68·9
	1587	188	138	73·4	1475	176	140	79·6
	1590	190	134	70·5	1487	171	125	73·1
	1591	185	142	76·7	1487	187	143	76·5
	1597	199	142	71·4	1508	173	125	72·5
	1602	186	145	78·0	1510	174	130	74·7
	1611	184	142	77·2	1510	177	133	75·1
	1616	196	139	70·9	1510	176	136	77·3
	1616	189	146	77·3	1512	181	134	74·0
	1626	193	137	71·0	1516	182	129	70·9
	1627	200	143	71·5	1520	182	126	69·3
	1635	192	132	68·8	1522	184	136	73·9
	1639	195	145	74·4	1525	175	138	78·9
	1644	187	142	75·9	1531	175	135	77·1
	1647	185	139	75·1	1533	175	134	76·6
	1648	172	128	74·4	1537	192	130	67·7
	1651	202	147	72·8	1538	186	135	72·6
	1651	198	146	73·7	1539	174	124	71·3
	1657	195	148	75·9	1540	178	136	76·4
	1663	186	140	75·3	1542	188	135	71·8
	1664	196	144	73·5	1548	186	131	70·4
	1671	195	146	74·9	1550	185	137	74·1
	1673	209	148	70·8	1553	175	138	78·9
	1674	194	146	75·3	1555	188	137	72·9
	1686	182	148	81·3	1555	173	131	75·7
	1689	197	150	76·1	1559	188	137	72·9
	1693	192	136	70·8	1560	184	138	75·0
	1693	190	143	75·3	1561	178	128	71·9
	1698	189	140	74·1	1561	188	138	73·4
	1705	180	145	80·6	1562	183	136	74·3
	1707	190	142	74·7	1563	181	132	72·9
	1708	194	147	75·8	1564	188	137	72·9
	1724	195	136	69·7	1564	183	138	75·4
	1730	205	143	69·8	1567	188	130	69·2
	1730	188	140	74·5	1580	186	137	73·7
	1731	203	143	70·4	1580	178	136	76·4
	1733	199	144	72·4	1582	182	142	78·0
	1740	197	153	77·7	1589	173	142	82·1
	1747	197	137	69·7	1592	179	132	73·7
	1761	199	136	68·3	1593	180	132	73·3
	1762	199	145	72·9	1594	182	132	72·5
	1768	197	146	74·1	1606	180	132	73·3
	1772	193	137	71·0	1609	192	145	75·5
	1779	199	146	73·4	1620	174	134	77·0
	1830	196	145	74·0	1622	192	136	70·8
	1833	201	130	64·7	1628	185	132	71·4
	1875	195	136	69·7	1644	174	132	75·9
	1876	201	146	72·6	1722	187	134	71·7
Means	1684·200	192·940	141·940	73·648	1551·460	181·420	134·380	74·134
Standard deviation	78·120	7·150	5·121	3·233	51·504	5·952	4·646	3·002
Corr. coeff. stat./H.L.		0·456 ($P<0·01$)				0·161 (n.s.)		
Corr. coeff. stat./H.B.		0·015 (n.s.)				0·053 (n.s.)		
Corr. coeff stat./C.I.		−0·373 ($P<0·01$)				−0·086 (n.s.)		

Jews born in Amsterdam and by Spier (1929) on Japanese born in America (calculated adult mean).

The outcome of the mass of confusing, and sometimes conflicting, evidence which has been published on this subject seems to be that a change in environment—a change usually for the better—may be sufficient to jolt the hereditary mechanism out of its accustomed groove. The alteration effected is not uniform: high indices are downgraded, low indices are upgraded, those in between are unchanged. Thus all tend to converge upon a common mean within a restricted range of about 80–82. Much remains to be done on this subject but the results to date suggest that a favourable environment induces a trend towards a common headform with an index at the lower limit of brachycephaly.

(6) *Relationship of ectocranial index to endocranial index*

Weidenreich (1945) could find no correlation between these indices in a series of twenty-one skulls he had measured. Harris (1926) records a similar discrepancy in gorillas. This line of investigation seemed worthy of further pursuit, however, and Weidenreich's series was extended by the addition of such data on twenty skulls in this Department. The information is given in Table 4. The figures for the two indices were then plotted (Fig. 2)—the ectocranial indices in ascending order, the corresponding endocranial indices on the same scale but in a separate curve. As is to be expected, the endocranial curve—despite occasional fluctuations—is generally above the ectocranial curve; and the discrepancy between the two diminishes fairly constantly from the longest skulls to the shortest. Most interesting, however, is that through a limited ectocranial range of about 79·8–82 the two indices conform much more closely than anywhere else in the series. While the number of observations is relatively small, and the concordance is far from exact, there is a clear indication that within this range the skull form approaches most closely to that of its contents.

(7) *Relationship of cranial index to cranial capacity*

Apart from cranial index, capacity depends upon such factors as height of skull, thickness and rounding of the bones, etc. and lies rather beyond the scope of this inquiry. Weidenreich (1945) denies any correlation between headform and capacity and quotes a number of examples to support his view; at the same time, there is some hint of such a correlation in the work of Bolk (1908) and Schwerz (1912). These workers both found that in a series of skulls the mean cranial capacity tends to a maximum in the upper mesocephalic range.

(8) *Stature*

A generalization known as 'Ammon's law' (Kappers, 1945) postulates a negative correlation between stature and cephalic index—the greater the stature the lower the index, i.e. the longer the skull. This has been confirmed by Pittard (1905), by Boas (1940) and, with some difficulty, by Kappers (1945).

The correlation was checked on the aborigine data collected by Burston (1913), Campbell & Lewis (1926) and Campbell & Hackett (1927). The results (Table 3) show that for the male aborigine the correlation coefficient for stature and cephalic index is −0·373; this is not a high correlation but, since the probability that it is due to chance is less than 1 in 100, it is significant. Similar

Table 4. *Ectocranial and endocranial indices of forty-one skulls; twenty-one measured by Weidenreich* (1945), *the remainder measured for this investigation*

Skull	Ectocranial index	Endocranial index	Authority
H. soloensis, V	66·3	76·2	Weidenreich
African	68·5	70·0	This paper
African	69·2	72·0	,,
Australian aborigine	69·5	74·4	,,
Australian aborigine	70·0	73·1	Weidenreich
Australian aborigine	70·3	79·5	This paper
African	70·4	71·8	,,
Chinese	70·5	74·3	Weidenreich
Sinanthropus, X	71·8	74·2	,,
Sinanthropus, XII	72·3	76·8	,,
H. rhodesiensis	72·3	78·8	,,
Sinanthropus, III	72·8	78·3	,,
Sinanthropus, XI	72·9	76·7	,,
African	73·5	71·7	This paper
H. neanderthalensis	73·8	78·6	Weidenreich
Ehringsdorf	74·0	78·8	,,
Ekhul, V	74·5	80·3	,,
African	74·7	79·5	This paper
La Chapelle	75·0	79·0	Weidenreich
Tamil	75·1	73·5	This paper
Chinese	75·2	75·6	,,
European	76·3	79·8	Weidenreich
Pithecanthropus, II	76·7	81·8	,,
European	77·1	80·4	This paper
Tabun, I	77·1	80·8	Weidenreich
Gibraltar	77·2	83·9	,,
European	77·4	76·3	,,
European	78·9	81·4	This paper
European	79·6	86·7	Weidenreich
African	79·8	79·6	This paper
F.T. European foetus	79·8	81·8	,,
African	80·0	79·6	,,
Chinese	80·3	81·6	,,
European	80·7	80·2	,,
European	81·8	81·8	Weidenreich
Chinese	82·6	84·4	This paper
European	82·8	86·7	,,
European	85·9	88·1	Weidenreich
Chinese	86·9	92·0	This paper
Chinese-Tamil cross	93·4	95·1	,,
European	94·6	95·0	Weidenreich

correlations were determined for stature/head breadth and stature/head length: there is no significant correlation with head breadth but the correlation with head length is of the same order as that with cephalic index.

The generalization holds weakly for the aboriginal male, then, and depends mainly upon head length. In the aboriginal female, however, there is no

significant correlation between stature on the one hand and head breadth, head length or cephalic index on the other (Table 3). Kappers (1945) was equally unable to establish any correlation between stature and cephalic index for the females of other groups.

Turning to mankind in general, similar calculations were made for the males of fifty other groups and the females of thirty-eight other groups (Table 5). In

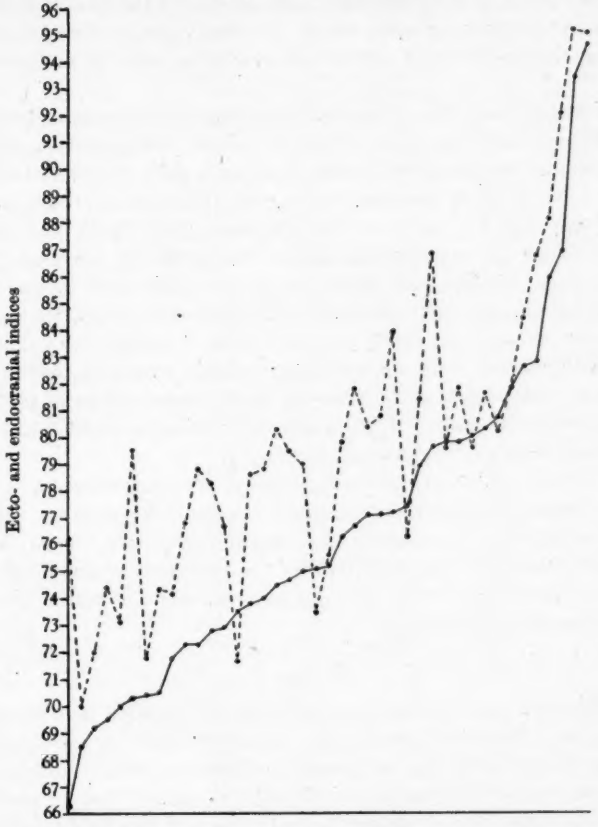

Fig. 2. Comparison of ectocranial indices (continuous line) with corresponding endocranial indices (broken line). Ectocranial indices in ascending order. Data: Table 4.

each case the figures quoted are group means derived from what seem to be the most reliable sources. In the males the correlation coefficient is 0·211, in the females 0·1321. Neither figure is statistically significant although the male correlation remains higher than the female, and the sign of the coefficient has changed—this, if it means anything, indicates some tendency for increasing stature to go with rounder-headedness, a tendency in harmony with what

appears to have been the course of human evolution generally (see Le Gros Clark, 1945). In any case, it is clear that 'Ammon's law' has significance only within a homogeneous group, a fact already insisted upon by Pittard (1905) and Boas (1940). The 'law' does not apply to females, probably because the shorter growing period does not permit such full skull differentiation as in the male: as Boas (1940, p. 48) points out, '...the most characteristic forms of each type are found in the adult male'. In the case of the skull it is clear that the additional lengthening upon which 'Ammon's law' is based is achieved late, during the few years of further differentiation after female growth has ceased.

Since increasing stature is, in general, associated with increasing head length it is to be expected that dwarf types, in whom differentiation stops prematurely, will be rounder-headed than those more fully differentiated. This is clearly so in the observations made on dogs by Weidenreich (1940) and in the human pygmy material collected by Kappers (1939, 1945), and the mean cephalic index for the pygmies (stature less than 1500 mm.) in Table 5 is 80·9 (range 79·0–83·0). In this connexion the females of the taller groups must be regarded as modified dwarfs. However, the association of rounder heads with shorter bodies is only part of the story, as Table 5 shows. For example, the Northern Andamanese, with a mean stature of only 1486 mm., have the same mean cephalic index (82·5) as the Sara who have a mean stature of 1817 mm.—the highest recorded. Moreover, the whole story of human evolution associates rounder-headedness with increasing stature.

Thus, there are two kinds of brachycephaly: one, characteristic of infants, dwarfs and females, represents incomplete general differentiation; the other, associated with tallness, expresses the human evolutionary trend. This was recognized by Macalister (1898) who called the two kinds primary and secondary brachycephaly respectively. Failure to appreciate this distinction has led to much unnecessary confusion.

(9) *Sex*

It is well known that females are usually rounder-headed than the males in the same group. This observation rests on ample evidence and depends, as already shown, upon the shorter period available for female differentiation. However, the mean male index does sometimes exceed that of the female. Martin (1928) doubts the accuracy of observations of this kind, and the only exception admitted by Kappers (1945) occurs in Armenians. At the same time, there is little justification for doubting figures simply because they conflict with an accepted hypothesis and the data employed here (Tables 5 and 6) are taken just as published.

At the outset, the relationship was tested on the skulls of Australian aborigines, fifty of each sex (Table 1). The mean index for the female (71·3) exceeds that for the male (70·2) by 1·1—a significant difference, for the probability that it is due to chance is only 1 in 20. The individual values were then plotted,

Table 5. *Stature and cephalic index of males of fifty peoples and of females of thirty-eight peoples*

	Male		Female		
People	Cephalic index	Stature (mm.)	Cephalic index	Stature (mm.)	Source of data
Veddah	70·5	1533	—	—	Haddon (1929)
Buduma	72·5	1755	72·3	1620	Martin (1928)
Kadir	72·9	1577	74·2	1430	,,
Australian Aborigines	73·7	1684	74·1	1552	Table 3
Bedawin	75·0	1660	—	—	Haddon
South African Bastards	75·8	1684	76·7	1570	Martin
American Eskimos	77·0	1577	74·5	1497	,,
Ainu	77·3	1567	78·4	1471	,,
Polar Eskimos	78·0	1570	77·4	1450	,,
Senoi	78·0	1520	—	—	Haddon
Batwa	78·1	1522	77·7	1437	Martin
Mataco	78·1	1638	77·8	1529	,,
M'Baka	78·1	1671	77·9	1568	,,
Chinese	78·3	1639	—	—	Haddon
Kamschatkans	78·5	1601	77·4	1499	Martin
Tunguse	78·7	1565	79·3	1465	,,
Semang	79·0	1528	—	—	Haddon
Ituri	79·0	1390	—	—	,,
Ostiaks	79·2	1563	79·0	1441	Martin
Mawamba Pygmies	79·5	1408	77·6	1356	,,
Tapiro	79·5	1449	—	—	Haddon
Fuegians	79·5	1577	78·9	1473	Martin
Shoshoni	79·5	1661	79·5	1528	,,
Fan	79·5	1698	80·5	1589	,,
Chiriguan	80·2	1634	81·4	1517	,,
Aueto	80·2	1581	81·8	1521	,,
Jukagires	80·4	1560	80·0	1470	,,
Asiatic Eskimos	80·7	1673	79·7	1518	,,
Danes	80·7	1691	81·5	1592	,,
Kalmuck	81·1	1634	82·6	1598	,,
Ossetes	81·5	1690	81·9	1573	,,
Lithuanians	81·5	1656	82·0	1546	,,
White Russians	81·5	1652	82·2	1544	,,
Polish Jews	81·8	1610	82·9	1506	,,
Aeta	82·0	1465	—	—	Haddon
Tschuktsches	82·0	1622	81·8	1520	Martin
Sara	82·5	1817	80·0	1676	,,
Northern Andamanese	82·5	1486	81·9	1385	,,
Polynesian	82·6	1720	—	—	Haddon
Jakutes	82·7	1624	80·9	1512	Martin
Southern Andamanese	83·0	1482	82·7	1402	,,
Balkan Tatars	83·3	1657	83·5	1546	,,
Hawaiians	83·4	1713	84·2	1626	Dunn (1928)
Samoyed	84·4	1550	83·9	1430	Martin
Pamiri	85·0	1683	—	—	Haddon
Tehuelche	85·0	1780	—	—	,,
Armenian	85·8	1685	84·1	1529	,,
Turki	86·0	1675	—	—	Haddon
Kirgish	89·4	1651	86·3	1503	Martin
Californian Indians	89·7	1709	89·9	1580	,,
Means	80·282	1614·74	80·22	1514·97	
Standard deviation	3·836	91·37	3·536	69·74	
Corr. coeff. stat./C.I.	0·211 (n.s.)		0·1321 (n.s.)		

Table 6. *Cranial indices of males and females of sixty-six peoples. Data from Martin* (1928) *except where stated otherwise*

People	Cranial index		Remarks
	Male	Female	
Pericues	66·1	68·5	—
Torres Straits	68·3	70·3	—
Fidschi Islanders	69·6	68·9	—
Loyalty Islanders	69·8	71·8	—
Australians	70·3	71·6	Martin, mean of three figures
Babase	70·4	71·6	—
Punjabi	70·7	72·3	—
Tamils	70·8	70·3	—
Duke of York Islanders	70·9	71·8	—
Papuans	71·0	73·0	—
Ambitle	71·1	71·1	—
Veddahs	71·2	72·7	Hill (1941)
Eastern Eskimos	71·3	71·4	—
New Caledonians	71·3	74·4	—
Hottentots	71·4	74·9	—
Greenland Eskimos	71·5	71·9	—
Sinhalese	72·5	72·0	—
Kaffirs	72·5	72·9	—
New Irelanders	72·9	75·1	Martin, mean of two figures
Naqada	73·2	74·6	—
Cuenca	73·2	75·0	—
Tutan	73·4	76·3	—
Germans	73·7	74·1	—
Friterpen	73·7	74·5	—
Tasmanians	73·9	75·9	Mean, Wunderly (1939), Hrdlička (1928)
Eskimos	74·2	74·9	—
Portuguese	74·3	75·6	—
Micronesians	74·4	75·0	—
Eastern Tschuktsches	74·4	76·5	—
English	74·7	75·0	—
Ama Zulus	74·8	72·9	—
Maoris	74·9	73·9	Mean, Martin, Turner (1884)
Anglo-Saxons	75·0	75·0	—
Marquesans	75·0	78·2	—
Thebans	75·1	76·5	—
Tahitians	75·1	77·4	—
Groterpen	75·4	77·1	—
Hawaiians	75·5	78·4	—
Teneriffians	75·9	77·5	—
Ainu	76·0	77·2	—
Quipuzcoans	76·2	77·6	—
Fuegians	76·9	77·8	—
Friesians	77·0	79·0	—
Californians	77·3	77·5	—
Guanches	77·3	78·9	—
Cretans	78·0	80·9	—
Japanese	78·3	79·7	—
Gallier	78·4	75·4	—
Etruscans	78·5	78·2	—
Asiatic Woguls	78·8	78·4	—
Prussians	79·2	78·9	—
Kanakas	79·3	80·3	—
Old Pompeiians	80·3	81·0	—
Andamanese	80·6	82·7	—
Italians	80·8	80·0	—
Alsatians	80·8	81·2	—
Bashkires	81·0	81·0	—

Table 6 (*continued*)

People	Cranial index		Remarks
	Male	Female	
Javanese	81·5	80·9	—
Kalmucks	81·6	81·0	—
Romanians	82·3	83·2	—
Wurttemburgers	82·6	82·7	—
Bohemians	83·1	83·5	—
Bavarians	83·8	84·2	Martin, mean of two figures
Swiss	84·4	84·7	—
Tirolese	85·0	84·9	—
Telengetes	86·0	87·3	—
Means	75·73	76·64	

each sex in a separate curve but to the same scale, in strictly numerical order (Fig. 3). Within the range of these observations (64·2–77·8) the curve for the female lies consistently above that for the male. Taken in conjunction with similar findings on aborigines by others, this indicates that the mean female cranial index really does exceed the mean male index.

The matter was then put to a general test by taking the mean male and female cranial indices of sixty-six other groups (Table 6): the difference between the mean female figure (76·64) and the mean male figure (75·73) is 0·9—quite comparable with that for the aborigines. Then similar curves for the two sexes were plotted. In the first place the male figures were plotted in strictly ascending order with the corresponding females in a separate curve on the same scale (Fig. 4). Despite some fluctuations, the female curve runs mainly higher than the male, the disparity diminishing progressively from the bottom to the top of the series. However, over the limited range of 78–82 the female fluctuations virtually cancel out and the two curves may be considered practically coincident. In this respect the similarity between this curve and that comparing ectocranial and endocranial indices (Fig. 2) is quite striking. To emphasize the sexual distinction the male and female figures were next plotted, independently of any racial affinity, each in strictly numerical order (Fig. 5). Now most of the fluctuations disappear and the female curve is more consistently above the male curve; there is still a progressive convergence from bottom to top, and the tendency to coincide in the lower 80 range is preserved. Comparison of Fig. 3, which represents individual aborigines, with Fig. 5, which represents means for different groups, reveals that the distinction really is one of sex: the aboriginal curve might very well have been taken from the lower part of Fig. 5, the indices simply do not go high enough to disclose the convergence found in the more extended series.

Elimination of the sexual distinction over the 78–82 range can be brought out more clearly, perhaps, by isolating this group and comparing the frequency with which the female exceeds the male and vice versa with the corresponding frequencies above and below this range (Table 7). It will be seen that while above and below the 78–82 range the female exceeds the male in over 85 % of

cases, within this range there is no difference between the sexes. Even allowing for the small number of observations, the correspondence at 78–82 is very striking.

A point of interest is that skull measurements illustrate the sexual difference much better than do head measurements. In the case of the cranial index of the aborigine (Table 1) there is a significant difference of 1·1 between the sexes, but with the cephalic indices (Table 3) the difference between female mean (74·1) and male mean (73·7) is only 0·4, which is not significant. Also, taking mankind in general (Table 6), the mean cranial index for females exceeds that for males by 0·9—comparable to the difference found in aborigines; but with the cephalic index (Table 5) the female mean (80·2) is practically the same as

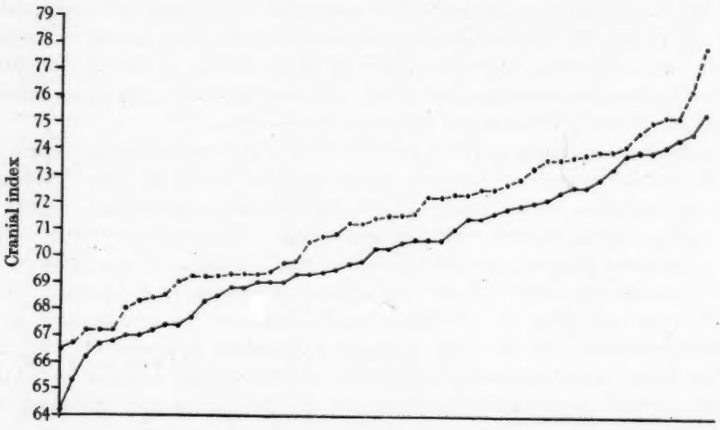

Fig. 3. Cranial indices of fifty male (continuous line) and fifty female (broken line) Australian aborigines. Each point represents an individual; each sex plotted in ascending order. Data: Table 1.

the male mean (80·28). It seems probable that reduction of the sexual difference in whole head measurements depends upon greater development of the temporal muscle in males, for Schultz (1940) found that such development in older chimpanzees adds considerably to the breadth of the head. In any case, the observation justifies Morant's (1928) preference for purely skeletal measurements.

As already noted, the generally rounder head in the female simply reflects a shorter period of differentiation. This, however, does not apply to such secondary sexual characters as prognathism for, according to Boas (1940), secondary sexual characters are present in childhood. Moreover, Huxley (1932) and Reeve & Huxley (1945) have shown that in other animals secondary sexual characters with a high growth ratio may impose a localized distortion upon the total growth gradient.

To summarize: the female is generally more brachycephalic than the male, a distinction which appears better from skull than from head measurements. The rounder female head is an example of primary brachycephaly—relative dwarfism or incomplete differentiation and, unlike prognathism, is not a secondary sexual character. The difference tends to vanish over a limited cranial index range of 78–82.

(10) *Individual development*

The fact that the child's head is generally rounder than the adult's is recognized in the term 'infantile' commonly applied to those with a high

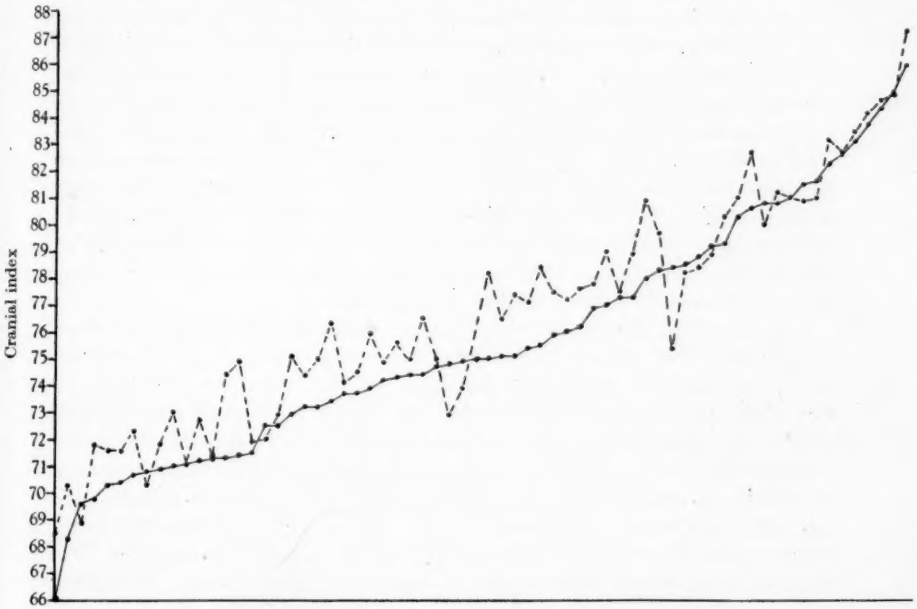

Fig. 4. Cranial indices of males (continuous line) and corresponding females (broken line) of sixty-six peoples. Each point represents a group mean. The males are plotted in ascending order. Data: Table 6.

cephalic index. However, while the rounder head is more like the infant's in many groups it is not so in all, nor is the process of head development from infancy to adulthood always comparable from one group to another.

The aboriginal skull collection in the Adelaide Museum contains one of a newly born foetus which, unfortunately, retains a good deal of birth moulding. As it is, the skull has a cranial index of 86, but even the most generous allowance for the distortion leaves the index still over 80. Starting from this point (Table 2), the index drops quickly to the lower 70's during the first few years

(Fig. 1), and then more slowly until about the age of 10; after a minor rise in early adolescence there is a progressive decline to the adult mean. Earlier cessation of growth puts the adult female mean naturally on the curve some way in front of the adult male mean. This is the type of curve usually visualized for dolichocephalic people.

However, in groups where the mean adult index is high, e.g. in the upper 80's, the curve of skull maturation is different. In these cases, according to Ranke (1905), Martin (1928), Klein (1935), Kappers (1945) and others, the cephalic index of the newly born is in the lower 80's, being less than the adult mean. Now, in development, there is a rise during the first 2 or 3 years to a level above the adult mean; after that there is a gradual fall, often with a slight elevation in early adolescence, to the adult standard. Thus, the mean cephalic index at birth is much the same for both the dolichocephalic and the brachycephalic: compare, for example, the cranial index of just over 80 for the aboriginal foetus with the mean cephalic index of 81·1 for the newborn of a predominantly brachycephalic group (Ranke, 1905). Aeby (quoted by Vogt, 1864, p. 30) confessed himself unable to distinguish the skulls of foetal negroes and Europeans, Macalister (1898) drew attention to the similarity of the foetal skulls of British, Egyptians and Hindus, and other examples could be quoted. It seems clear that no matter what may be the mean cephalic index of the parents, the mean index of the newborn falls within a very restricted range which lies close to the lower limit of brachycephaly.

This view was checked on the heads of thirty-nine European infants—sixteen males and twenty-three females, aged from 1 to 22 days and without head moulding. The mean cephalic index for the males was 78·39 (range 74·3–82·6), the mean for the females was 78·4 (range 73·6–84·2). Sexual difference is negligible and the combined mean is 78·4. To this may be added a mean of 82·0 for five full-time, undeformed formalin-fixed European foetuses. In addition, Ranke (1905) has published figures for twenty European infants (age 1–21 days): eleven males gave a mean index of 81·5, nine females a mean of 80·7; again the sexual difference is slight and the combined mean is 81·1. The mean of the sixty-four observations on Europeans recorded here is 80·5. This may be compared with the cephalic index of 76·6 (well within the European range) of the part-aboriginal infant omitted from the above series, with a cranial index of 80+ for the full-time aboriginal skull and with a mean index of 81·1 for three European foetal skulls.

These observations all combine strongly to support the view that in the matter of headform there is a foetal norm, restricted to a small range of indices about the lower 80's, which seems to be common to the most diverse groups and to both sexes. This may be considered the primary norm for the whole human species. Whether or not this holds for other primates is yet to be determined.

It appears further that where the mean adult index lies clearly below or above the primary norm a major characteristic deviation in the adult direction

occurs during the first 2 or 3 years of post-natal life. This establishes a secondary, or group, norm from which there is a gradual decline to the adult mean. Skull differentiation thus shows four distinct stages: a common human stage at birth, a characteristic group stage at 2–3 years, an adult female stage in late adolescence and an adult male stage at the end of growth. In other words, common human heredity determines headform until birth, specific group heredity comes into play during the first few years, and the sexual factor determines the time at which skull differentiation ceases. Davenport (1926) has drawn attention to a tremendous acceleration of growth in the period immediately after birth—this appears to coincide with the attainment of

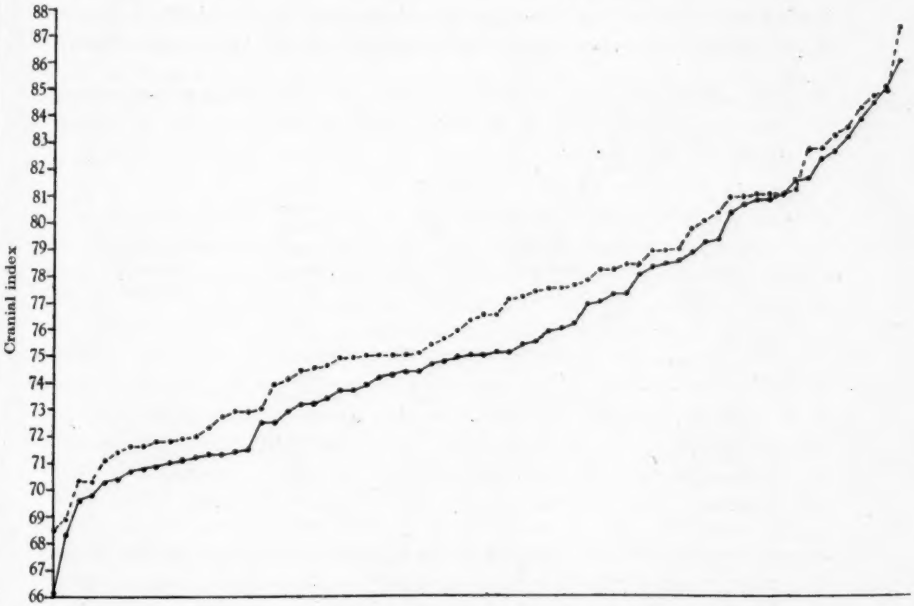

Fig. 5. The same data as in Fig. 4 but the two sexes are now plotted independently, each in ascending order of cranial index. Males (continuous line), females (broken line).

specific headform; he also mentions a lesser acceleration during early adolescence which agrees with the observed minor increase in cephalic index at the same time. At all events, specific group headform is already determined by the third year and the change thereafter may not be particularly striking. This is probably why Pearson & Tippett (1924), whose series started with 5-year-olds, could find no significant change in headform during ontogeny. It must be remembered, too, that even the changes of the first 3 years will be clearly marked only in groups which show a decided adult deviation from the primary norm, and it is evident that while the term 'infantile' can be applied to the

rounder-headed members of dolichocephalic groups it should not be used for the rounder-headed of brachycephalic groups where the infant's head is longer than the adult's. Finally, since the adult female clings to a juvenile form of skull it is easy to see why disparity with the male disappears when the adult male index falls within the foetal range of 78–82.

All this makes it clear that both the long headed and the short headed are divergent from the primary human norm. In other words, they are both equally specialized, or differentiated—but in opposite directions. Wood Jones (1931) has pointed out that many of the characters which early man shares with apes and monkeys, usually designated 'primitive', really represent differentiation away from the primitive form as found in the foetus, and he calls them 'pithecoid specializations'. These include dolichocephaly. Actually, a similar observation was made long ago by Aeby (quoted by Vogt, 1864, p. 31): 'I have

Table 7. *Analysis of data in Table* 6 *to show the distribution of female-male excess of cranial index in the* 78–82 *group as compared with the groups above and below this range*

Range	Female exceeds male	Female equals male	Male exceeds female	Totals
66·0–77·9	38 (84·44%)	2 (4·44%)	5 (11·11%)	45 (99·99%)
78·0–81·9	6 (42·86%)	1 (7·14%)	7 (50·00%)	14 (100·00%)
82·0–87·9	6 (85·71%)	0 (—)	1 (14·3%)	7 (100·00%)
Totals	50	3	13	66

Further analysis of same data

Range	Female equals or exceeds male	Male exceeds female	Totals
66·0–77·9	40 (88·90%)	5 (11·10%)	45 (100·00%)
78·0–81·9	7 (50·00%)	7 (50·00%)	14 (100·00%)
82·0–87·9	6 (85·70%)	1 (14·30%)	7 (100·00%)
Totals	53	13	66

already elsewhere drawn attention to the similarity of all foetal cranial forms. I am now prepared to lay it down as a general law, that a cranial form occupies a higher rank accordingly as it advances by uniform peripheral development from the foetal form; and that it stands lower, accordingly as the growth is confined to certain directions and points. From this point of view, the narrow skull must be considered as a lower type....' If 'higher' is replaced by 'primitive' or 'undifferentiated', and 'lower' by 'specialized' or 'differentiated', Aeby's conclusion is virtually the same as that reached here. And he continues (loc. cit): 'We will not leave unmentioned that possibly the same position may be assigned to the most decided broad skulls.' This could be considered an anticipation of the views presented here regarding the higher grades of brachycephaly.

The truly primitive skull—the primary, or foetal, type—has an index close to the lower 80's, at the lower limit of brachycephaly as usually defined. The

adult skull may not differ much from this, or it may diverge, becoming either longer or shorter. Thus adult skulls fall into three groups: dolichocephalic, foetal and brachycephalic. The index range called 'mesocephalic' is only a degree of dolichocephaly and has no biological significance. 'Mesocephaly' could be applied to the only truly intermediate range—the foetal standard of 78–82, but long usage otherwise would render such change impracticable. The term is better abandoned: then, brachycephalic skulls are those with an index over 82, dolichocephalic skulls have an index under 78, while skulls with an index between 78–82 should be designated 'paedocephalic'.

(11) *Independent variation in the skull*

Evidence has already been adduced to show that the jaws and the cranium vary independently. To this may be added Begg's (1939) finding that jaw size and tooth size are inherited independently. Other examples of independent variation are head length and head breadth (Weidenreich, 1945) and, probably, head height as well (see Macalister, 1898). Attention may now be drawn to the probability that the front and the back of the cranium also vary independently.

A dolichocephalic skull such as that of the Australian aborigine exhibits frontal characters which are obviously far differentiated from the foetal type; brachycephalic skulls, on the other hand, tend to retain the foetal form in this region. But at the back of the skull things are otherwise. Here the dolichocephalic skull protrudes to an opisthocranion well behind the inion, and the expanse from foramen magnum to inion is long and comparatively flat; this is as in the foetus. In the highly brachycephalic skull, however, the maximum occipital protuberance usually coincides with the inion—whence the back ascends abruptly to the vertex, and the expanse between foramen magnum and inion is comparatively short and strongly curved; this is quite unlike the foetus. Thus, the dolichocephalic skull differentiates by frontal extension, the brachycephalic by occipital shortening; there may be all intermediate grades but there is no apparent correlation between the two.

DISCUSSION

It has been shown that headform in various groups is influenced by evolutionary trends, by heredity and probably environment, to some extent by stature and sex, and by individual development. There may be a relationship between headform and cranial capacity, but none could be found between headform and size of jaws. There is substantial evidence that some parts of the skull vary independently.

This survey has uncovered an interesting series of coincidences. The discrepancy between ectocranial and endocranial index is at a minimum over a limited range of 79–82. The sexual disparity in cranial index tends to disappear over a similar range of 78–82. In a favourable environment dolichocephals and brachycephals seem to converge upon a common mean of 80–82

in the first new generation. It is possible that the maximum cranial capacity lies close to the same range. And, finally, the mean cranial or cephalic index in the newborn of any group—irrespective of parental index—lies close to 80. These points have all been discussed in the appropriate place; put together thus they emphasize a common norm of 78–82, the point from which skull differentiation sets out and towards which, it seems, evolution is tending more and more to return. All this, together with the increasing persistence of such characters as metopism (Bolk, 1921), thinning of cranial bones and delay in suture closure (Weidenreich, 1940), might well be considered an example of Bolk's 'foetalization'—a progressive tendency to cling longer to the undifferentiated condition of the foetus. However, even if the foetal shape is retained, the skull does show quite a considerable change in size.

Males within a homogeneous group show some negative correlation between stature and cephalic index. This correlation does not extend to females or to mankind in general, nor is it evident in human evolution which associates increasing stature with progressive shortening of the head. Increasing stature differentiates away from the foetal norm and is a foetal tendency only to the extent that it may betray persistence of the foetal habit to increase in size. On the other hand, expanding bodily bulk has characterized the history of most of the successful animals for which there are adequate records. Such progressive increase has been attributed by some to 'orthogenesis', but since the existence of this agency has been called into question it seems preferable to seek a more satisfactory explanation.

Selection is unlikely to preserve either foetalization or orthogenesis for its own sake: some advantage must accrue and it is necessary to find where this advantage lies. A clue appears in the relation between ectocranial and endocranial index; it will be recalled that the disparity between the two is largely eliminated over the foetal index range. In the foetus the skull is little more than an additional cerebral membrane, conforming very closely to its contents. Adult skulls which preserve the foetal index similarly conform more closely to the brain than do skulls elsewhere on the index scale. The foetal pattern, then, provides the most economical kind of brain protection—a thin rigid cover with the least expenditure of material. The further possibility that this pattern affords the greatest capacity should also be taken into account. The advantages of retaining the foetal form of skull are manifest; the fact that this entails retention also of such other foetal characters as metopism, thinner bones and delayed suture closure may enhance the advantage or may be purely incidental. Certainly, persistence of these features does not contribute to further cerebral expansion, for von Bonin (1934) has shown that phylogenetically the human brain has betrayed no increase in size for a very long time.

According to von Bonin the size of the brain has become stable. He believes that increasing brachycephalization connotes internal reorganization, and that this has positive selective value. Here it is suggested, instead, that the factor which has selective value is the progressive tendency to maintain a form

of skull which may afford the maximum capacity in the most economical fashion.

It seems that while the mean human cephalic index shows an evolutionary rise, this does not imply that evolution has an extreme grade of brachycephaly as its ultimate aim. Excessive brachycephaly is as aberrant and specialized as excessive dolichocephaly and, under favourable circumstances, shows a tendency to drop to a lower grade. The thesis advanced here is that the whole evolutionary trend is towards securing a mean common index of 78–82. This in itself would ensure a rise in mean index for the mean of all the data in Table 6 is still only 76·64 for the female and 75·73 for the male, and the number of dolichocephalic groups greatly exceeds the number of brachycephalic. The advantages of preserving this form of skull have been fully discussed.

SUMMARY

1. The problem of headform has been considered in relation to evolution, race, jaw development, heredity and environment, internal form and capacity, stature, sex, and individual development. Data on the Australian aborigine were employed as a check on as many aspects of the investigation as possible.

2. The evolutionary record shows a progressive increase in mean stature together with a progressive rise in mean cephalic index.

3. Headform is a feature of race only because isolated groups, inbreeding for long periods, attain stable and characteristic means.

4. There is no significant correlation between headform and size of jaws in either sex. However, the rounder-headed female is significantly more prognathous than the male, and this is a secondary sexual character.

5. Headform is probably determined by so many independent variables that solution on simple Mendelian lines is very difficult. Since stable forms are preserved by isolated groups, however, hereditary transmission must play a part in maintaining them.

6. The available evidence suggests strongly that change to a more favourable environment tends to bring all indices to a common mean of 80–82.

7. The endocranial index is usually higher than the ectocranial, particularly in long skulls, but the difference practically disappears over a range of 79–82.

8. There is some suggestion that maximum mean cranial capacity is associated with a mean index just under 80.

9. There is a moderate negative correlation between stature and cephalic index for adult males in homogeneous groups. Females do not differentiate far enough to attain this correlation, nor is it found in mankind generally or in human evolutionary history.

10. Brachycephaly is found to be of two kinds: primary brachycephaly—characteristic of infants, dwarfs and females—is an expression of incomplete differentiation; secondary brachycephaly—associated with increasing stature—reflects the trend of human evolution as a whole.

11. Incomplete differentiation usually leaves the female skull rounder than

that of the male, particularly in long-headed peoples. The disparity practically disappears in the 78–82 index range.

12. Irrespective of the shape of the parental head, the newly born have a common mean index range of 78–82. Dolichocephalic groups show a fairly progressive fall to the adult mean; brachycephalic groups show a rise during the first 2 or 3 years to a level above the adult mean, and fall progressively thereafter.

13. Four stages in skull differentiation are observed: (*a*) at birth, the primary—or universal human—mean of 78–82; (*b*) during the first 2 or 3 years, a secondary—or specific group—mean; (*c*) in late adolescence, the adult female mean of incomplete group differentiation; (*d*) finally, the adult male mean of complete group differentiation.

14. Sexual skull disparity disappears in the range 78–82 because here the male approaches the 'infantile' condition retained normally by the female.

15. The foetal form of skull conforms most closely to the brain; the difference between ectocranial and endocranial index disappears over the foetal range for the same reason.

16. Adult human skulls may retain the foetal form or they may become either longer or shorter. The long skulls and the short skulls are both equally specialized away from the primitive foetal form.

17. The proper index grouping for skulls is: brachycephalic—over 82; dolichocephalic—under 78; the intermediate, or foetal, range of 78–82 should be called *paedocephalic*. This grouping has a definite biological meaning.

18. Cranial evolution is not tending towards a high degree of brachycephaly as such. The trend is more and more to preserve the foetal mean index of 78–82, and this in itself is sufficient to raise the whole human mean. This trend is not an example of 'foetalization' *per se* but reflects an evolutionary urge towards the form of head which seems to offer the greatest capacity at the minimum cost.

I am indebted to the Director of the Adelaide Museum for affording access to its unparalleled collection of aboriginal skulls; to the Director of Obstetrics, Adelaide University, and the Matron of the Queen Victoria Maternity Home for their co-operation in securing the head measurements of the infants; and particularly to Mr Cornish, Lecturer in Statistics, Adelaide University, for the time he has given to analysis of the data. To all of these I wish to express my deepest gratitude.

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OBSERVATIONS ON MUSCLE-FIBRE STRUCTURE

THE SWELLING OF MUSCLE FIBRES BY ACIDS AND ALKALIS

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INTRODUCTION

The work reported in this paper originated in an attempt to apply the method of microdissection to the problem of muscle-fibre structure. Remarkably little work has been done on muscle using this technique, and the few results that have been obtained are somewhat contradictory. A great deal is known about the behaviour of whole muscles under various conditions (see Fenn, 1945) and in recent years the ultrastructure of muscle has been investigated by means of polarized light (Fischer, 1936, 1944; Buchthal & Knappeis, 1938), electron microscopy (Hall, Jakus & Schmitt, 1946) and X-ray diffraction methods (Astbury, 1939; Spiegel-Adolf, Henney & Ashkenaz, 1944). Attempts have been made to correlate the results of these investigations with the remarkable properties of myosin solutions and myosin fibres (Engelhardt & Ljubimowa, 1939; Szent-Gyorgyi, 1942; Dainty, Kleinzeller, Lawrence, Miall, Needham, Needham & Shen, 1943). On the other hand, little attempt has been made to correlate our knowledge of myosin and muscle ultrastructure with the microscopic and macroscopic appearance and behaviour of muscle fibres. It was hoped that microdissection, combined with various chemical and physical reagents, would make such a correlation possible. In attempting to perform any micromanipulative operation, such as a micro-injection, on a muscle fibre, one soon comes up against a difficulty. The muscle substance tends to react to a variety of injuries in a characteristic way, i.e. by forming a retraction clot. The cross-striated appearance is lost locally, and a number of sarcomeres appear to run together, forming a dark, solid-looking mass which tends to retract from the sarcolemma and may progress down the fibre involving more and more sarcomeres. Such clots are no doubt similar to those described by Zenker (1864) in cases of typhoid fever, and the appearance is usually described as Zenker's waxy degeneration. The formation of retraction clots is in fact a very general reaction of a muscle fibre to a variety of injuries, for example, mechanical injury (Thoma, 1906), electrical injury, large doses of contracture-producing drugs such as caffein, devascularization (Le Gros Clark & Blomfield, 1945) and burns (Barer, unpublished). An excellent description of the formation of retraction clots in the living frog tadpole has been given by Speidel (1939). The formation of a retraction clot is, of course, a serious hindrance to microdissection, and an attempt was made to prevent the formation of such clots. The possibility that muscle clotting might resemble blood clotting in more than name alone led to the trial of anticoagulants such as

heparin, oxalates and citrates, but without success. A weak solution of citric acid was then tried, and this caused such interesting structural changes in the muscle fibre as to merit further investigation.

MATERIAL AND METHODS

Most of the work was done on teased frog muscle, but the striated muscle of toads, newts, rabbits, mice, rats, hedgehogs, guinea-pigs, man and insects was also used. For the most part fresh unfixed muscles were removed under anaesthesia or as soon after death as possible, transferred to a Ringer or saline solution suitable to the particular species, and were teased rapidly but as carefully as possible. After a period varying from a few moments up to 6 hr., in order to determine whether different periods of equilibration of the fibre had any effect on the changes observed, the teased fibres were transferred to a film of Ringer or saline on a $2\frac{3}{8} \times 1\frac{3}{8}$ in. cover-glass. The cover-glass was then inverted over a simple type of moist chamber on the microscope stage, allowing a micro-instrument to be introduced at each end of the chamber. In some cases the excitability of the fibres was tested by direct observation of their response to galvanic stimulation applied by two non-polarizable silver-silver chloride microelectrodes.

The micromanipulator. The type of micromanipulator used was that designed by Dr Schuster and described by him in the Appendix to this paper. The great advantage of this apparatus is its simplicity. It can be made in a laboratory workshop within a few days and is very easy to use. Movements in any direction in space can be made smoothly and continuously without letting go of the single control lever. This is a great improvement on instruments which require movement of a different control for each direction. As regards delicacy of control, this depends largely on practice. Most routine work was done using a $\frac{1}{6}$ in. objective, at a magnification of about 450 times. There is no doubt, however, that the instrument is capable of being used with $\frac{1}{12}$ in. oil-immersion objectives. Simple operations such as enucleation of amoebae are readily carried out, and can be performed in class by students with very little practice.

The instrument is also ideal for semi-microscopic work, such as accurate placing of electrodes on tissues or injection of small blood vessels. The working part can be removed from its stand and attached to a sliding bar held in a clamp and orientated in any desired direction. Microneedles and pipettes were made from soft glass, either drawn by hand over a microflame or else drawn on a special gravity control machine to be described elsewhere.

RESULTS

The following description applies to vertebrate striated muscle only. Insect muscle behaves generally in a similar fashion, but the differences in structure are sufficient to need a separate description elsewhere. It may be said at once that, apart from certain minor species differences, all vertebrate striated muscle fibres examined reacted in the same way to acids and alkalis. No major

differences in behaviour were found in muscles from widely different parts of the body. Fibres from the human eye muscles, for example, behaved very similarly to those from the sartorius of the frog.

When teased muscle fibres are treated with a little dilute acid, e.g. 0·2 % lactic acid, a number of interesting changes occur. Within a few seconds some of the fibres become less opaque, but the nuclei, which are never normally visible in a fresh fibre, become visible. The fibres frequently undergo a rapid initial shortening, but then gradually lengthen. Very often the change in opacity, accompanied by the appearance of the nuclei, seems to pass down the fibre like a wave, and sometimes the process stops short so that only part of a fibre may be involved. The decrease in opacity is accompanied by a loss of birefringence, which may also extend down the fibre like a wave. These are the earliest changes to be seen. In some fibres, however, the sarcolemma is seen to give way suddenly and knuckles or loops of muscle substance emerge. Fig. 1* shows such an appearance in a frog's muscle fibre. The nuclei are clearly visible, and a mushroom-like herniation of the muscle substance has occurred through a small gap in the sarcolemma. In favourable cases it is possible to follow the flow of muscle substance through such a gap. Nuclei and striations flow out at a variable rate, often too fast to follow, but sometimes quite slowly. Flow may occur from one side of the gap or from both sides. In Fig. 1 flow has occurred symmetrically from both sides, and the muscle substance has retracted away from the sarcolemma opposite the gap, leaving a clear V-shaped space. The way in which the sarcolemma ruptures is interesting. In what may be termed a lateral hernia, i.e. a herniation which occurs somewhere in the length of a fibre as in Fig. 1, the sarcolemma always presents a clear, sharp, rounded edge. This is well seen in Fig. 1 and in Fig. 2, which shows a small lateral hernia through a large circular tear in the sarcolemma. In fibres which have been cut in the process of teasing, one frequently sees what may be called a 'terminal hernia'. The muscle substance flows out of the cut end of the fibre, expanding considerably in diameter. The torn sarcolemma in such cases presents a sharp retracted edge (Fig. 3) which is frequently rolled upon itself. Fig. 4 shows a terminal hernia in which the entire muscle substance has been extruded, leaving an empty sarcolemmal sheath, the ends of which are rolled back. It will be seen that apart from a little debris the sarcolemma is empty and apparently structureless. Both terminal and lateral hernia may occur in the same fibre. Fig. 5 shows such a case, in which the sarcolemmal tear in the case of the lateral hernia is seen from above. Occasionally a lateral tear increases in extent until the entire fibre is encircled. When this happens, the sarcolemma appears to retract, leaving a swollen length of bare muscle substance, bordered by a curled-up retracted rim of sarcolemma on each side. Several such appearances may occur in the same fibre (Fig. 6), presenting a picture very reminiscent of the swelling of collagen (Kaye & Lloyd, 1924) or cotton fibres (Hock, 1942). The site of herniation in any fibre is quite unpredictable. If the fibre has a cut end,

* Pls. 1–4 contain Figs. 1–26.

terminal herniation usually occurs to a greater or less extent. Lateral herniae may occur anywhere. They may be very numerous in some fibres, but absent in others. If more than one hernia occurs in a given fibre, the muscle substance may be presented with alternative directions in which to flow. The direction of flow may become reversed or flow may cease. Occasionally the muscle substance becomes disorganized and torn. It is possible that lateral herniae occur at sites at which the sarcolemma was injured during teasing. In order to test this the sarcolemma was deliberately injured by means of a micro-needle. Such sites of injury did not appear to be specially prone to herniation on addition of dilute acid. If, however, the sarcolemma was punctured after addition of the acid, the muscle substance escaped as if under pressure, forming a typical lateral hernia. However, such artificially produced herniae were usually small and never reached the same extent as the spontaneous herniae (Fig. 7). The fact that multiple herniae may occur in the same fibre makes it unlikely that the site of a hernia is related to the point of entrance of a nerve fibre.

Effect of various acids

There seems no doubt that these phenomena are the results of a colloidal swelling of the muscle proteins by acids. Although first observed with citric acid, very similar effects were obtained with certain concentrations of lactic, formic, acetic, oxalic, tartaric and other organic acids, as well as with hydrochloric, nitric and sulphuric acids. There seems little doubt that the main effects are due to hydrogen ions. Specific anionic effects, if they exist at all, are not marked. The concentration of acid used is of some importance. By observing a number of teased fibres under the influence of various strengths of acid, it is possible to form a rough estimate of the extent of the effects, by taking into account such things as the number and size of the herniae and the rapidity of flow of muscle substance. Such estimates are of course quite arbitrary and subjective, but with practice it is usually possible to say whether one concentration of acid produces more swelling than another. In this way it can readily be observed that concentrated acids are far less effective as swelling agents than more dilute ones, but very dilute acids are again ineffective. In other words, there is an optimum concentration of acid for the production of swelling. To take a specific example, with frog muscle 0·1–2·0 % lactic acid produces good flow with extensive herniation. 5–10 % lactic acid produces fewer herniae and slower flow. 0·005 % acid produces a mere trace of terminal herniation. Citric acid acts best at a concentration of about 1·0 %, while hydrochloric acid and sulphuric acid act best at about 0·02 %. In general, organic acids appear to be more active than the mineral acids. Thus large lateral herniae are rather uncommon with hydrochloric or sulphuric acids. In other respects, however, the actions are similar.

Swelling of a tissue may manifest itself in two ways, by increase in weight and by increase in volume. No attempt has been made to measure the change in weight of single muscle fibres after swelling in acid, but increases in weight

of whole muscles under such conditions have been recorded (Loeb, 1897; Lloyd, 1916). As regards change in volume of single fibres, this is difficult to determine accurately. In some cases a fibre may show little increase in diameter after treatment with acid. This is easy to understand, as muscle substance may have escaped into herniae as a result of swelling, leaving less muscle substance to occupy the same volume. It must be remembered, also, that, assuming the length is unaltered, the volume of a cylinder varies as the square of its diameter. Thus a 10 % increase in diameter means a 21 % increase in volume. A 30 % increase in diameter corresponds to a 69 % increase in volume, and so on. In other words, what appears to the eye as a relatively small increase in diameter may correspond to quite a large increase in volume. In practice, increases of diameter up to 50 % are not uncommon. The herniated muscle substance is usually grossly swollen. This is seen particularly in terminal herniae, in which the diameter of the herniated cylinder may be two or three times that of the muscle substance still enclosed by sarcolemma (Fig. 17), i.e. the volume has increased from four to nine times that of the already swollen fibre, which is a considerable degree of swelling.

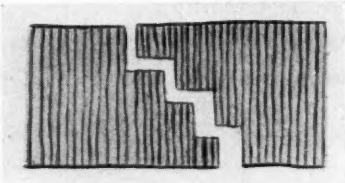
Microdissection of swollen muscle

The presence of a sarcolemma, which behaves as a tough elastic membrane, makes the microdissection of a fresh normal muscle fibre very difficult. In a brief unillustrated note Ensinger (1938) has claimed to be able to dissect out individual myofibrils. Chambers, however (reported in Speidel, 1939), has only been able to peel off fibrils of greatly varying sizes and thinks there is no very definite diameter for a myofibril. My observations on normal untreated fibres agree with those of Chambers in that I have found it extremely difficult to dissect out any fibrils of uniform diameter, and it might almost be concluded that individual myofibrils do not exist. However, there is good evidence based on direct observations that myofibrils do exist and are capable of independent contraction (unpublished observations). The reason why they are so hard to dissect is probably that they are embedded in a highly viscous matrix, the sarcoplasm, which makes them stick together in irregular groups.

The herniated muscle substance has the consistency of a viscous jelly. Strands of muscle substance frequently adhere to the microneedles, and it is very difficult to get rid of them. In some animals, notably rabbits and rats, it is possible to pull out very long loops of muscle substance through the hole in the sarcolemma (Fig. 8). Such loops exhibit a high degree of elasticity both longitudinally and transversely. No quantitative measurements have yet been made, but it can be stated that as a rule it is possible to stretch the muscle substance to more than twice its length or width, and on release it returns to its resting dimensions. Fig. 9 shows a loop of rabbit muscle substance which is being stretched by two microneedles outside the field of the photograph. It will be noted that on the whole the alignment of the cross-striations is good and they extend more or less completely across the fibre. Some lack of alignment of

myofibrils may occur at very high extensions. Fig. 10 shows the same region of the fibre on release of tension. It is difficult to believe that this is in fact the same fibre. The loop of muscle substance has become rounded, and it was unfortunately not possible to get all the striations in correct focus simultaneously. Nevertheless, it is clear that the striations are very much closer together, giving some indication of the degree of elasticity which the muscle substance must possess. The birefringence of the herniated muscle substance was not affected by stretching in various directions. It appears that acid swelling causes an irreversible loss of birefringence which is unaffected by local strains. It is not clear whether this is due to a specific action of the acid on myosin or to the increased absorption of water. A similar loss of birefringence was found by Kaye & Lloyd (1924) in swollen collagen.

If the herniated muscle substance is stretched beyond certain limits it tears in a manner depending on the direction of stretching. If stretched transversely, longitudinal splitting into groups of myofibrils may occur. As is the case with fresh muscle fibres, individual myofibrils are difficult to demonstrate owing to



Text-fig. 1. Diagram showing how a swollen muscle fibre may rupture when pulled longitudinally between two microneedles. Compare with Fig. 24.

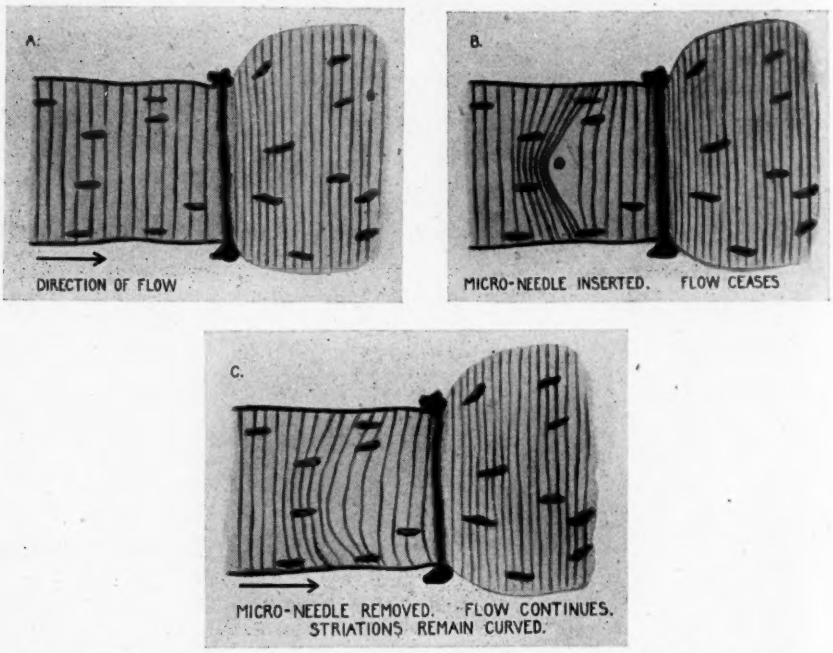
the stickiness of the sarcoplasm, but groups of myofibrils can be split into smaller and smaller units much more easily than with fresh muscle, since retraction clotting does not occur. Fig. 11 shows a swollen fibre in course of microdissection. Several strands of myofibrils are seen. Many narrow strands of myofibrils can be seen, and even the finest of these is clearly striated. Fig. 12 shows a few of these strands under higher power, and again the smallest fibrils, 2–3 μ in diameter, are striated. Stretching in the direction of the fibre axis tends to cause transverse splitting. Quite often the break does not occur cleanly but in a series of steps (Text-fig. 1). This is probably due to tearing of different groups of myofibrils at different levels. In small mammalian fibres, however, it is sometimes possible to cause the fibre to break across cleanly and, in fact, to tease out individual sarcomeres in the form of thin flat discs extending right across the fibre. Such discs are probably identical with those described by Bowman in 1840 and known as Bowman's discs. It is possible to turn these discs around by means of microneedles and to study them in profile and full face. They appear to be composed of short lengths of groups of myofibrils. As far as can be judged these lie in a semi-fluid matrix and do not appear to be rigidly connected by any special cementing substance. The existence of these

discs is a matter of such importance for theories of muscle structure that it is hoped to investigate them in more detail using more refined apparatus. In frog muscle the fibres are generally too wide and the striations too close together to allow them to be split easily into discs.

The behaviour of the cross-striations during swelling is very interesting. If herniation occurs, the contents of the fibre flow towards and through the rupture in the sarcolemma. Unless the muscle substance becomes grossly disorganized the various elements present their usual relationships. Thus nuclei and striations flow out together and the alignment of the striations is maintained. After escaping from the sarcolemma the width of the muscle substance and of the striations also increases. In the case of lateral herniae, the striations become curved and may exhibit most beautiful patterns. Such 'finger-print' distortion of striations is well seen in Fig. 13, which shows a mass of herniated muscle substance from a rabbit. The site of the hernia itself is not shown. Fig. 14 shows a lateral hernia in which the length of the striations has greatly increased and some bending has occurred near the edges of the hernia. Some of the striations can be followed right across from the edge of the hernia to the opposite side of the muscle fibre, showing that the myofibrils must be perfectly aligned. Thus, at least after treatment with dilute acids, the striations do not behave as rigid structures but are capable of undergoing considerable bending. Further evidence on this point is obtained by microdissection. If a microneedle is inserted into the swollen muscle substance, either herniated or still within the sarcolemma, the striations can readily be indented and stretched and behave like viscous elastic bodies. The same effects can be obtained on fresh untreated fibres in favourable cases, before retraction clots form, and also in reconstituted frozen-dried fibres.

If a microneedle is thrust up through a muscle fibre in which flow of striations is taking place in such a way that the advancing striations impinge on the needle, the first striation gets held up at its middle (Text-fig. 2), but the extremities of the striation tend to get carried on a little way so that the striation appears curved in parabolic form. The striations immediately behind get crowded together and also become curved. In a few seconds the flow ceases. If now the microneedle is suddenly withdrawn, flow may continue, but those striations which have become curved remain so and flow on in this form. Thus the evidence obtained from microdissection leads to the conclusion that the striations behave neither as solid bodies nor as a pure fluid, but rather as if composed of a viscous gel, which can be readily deformed. The observations of Kühne (1863) are often quoted as evidence of the fluid or thixotropic nature of the striations. This author observed a living nematode worm moving within a muscle fibre. He claimed that the striations parted to allow the worm to pass through, and reformed when it had done so. This has been taken to mean that the striations are fluid in nature or else are composed of a gel which becomes fluid on mechanical agitation (thixotropic gel). Speidel (1939) has criticized this interpretation, and has suggested that the worm was actually moving in the fluid sarcoplasm, and

not through the myofibrils themselves. This explanation seems to be a very likely one and fits in with my own microdissection observations. It is not possible to move a microneedle freely through the striations, nor do the latter become liquefied by mechanical agitation. It is, of course, true that most of these observations were made on muscle fibres which had been treated with various chemical reagents and in which the striations might no longer be in their normal state, but the same results were obtained in reconstituted frozen-dried fibres



Text-fig. 2. Diagram showing the behaviour of the cross striations during flow. A, normal flow. B, the tip of a microneedle has been pushed up between two advancing striations. The striations become bent and flow ceases. C, microneedle removed. Flow continues but the striations remain bent.

and, in favourable cases, in fresh living fibres. An attempt is being made to repeat Kühne's observations by injecting living motile bacteria and Infusoria into single muscle fibres.

Observation of the flow of striations has yielded no evidence in support of the spiral theory of muscle structure (Tiegs, 1934). If the striations were arranged as a spiral instead of as discs it might be expected that some sort of 'uncoiling' of the spiral would occur under some conditions of flow. This has never been seen in vertebrate muscles, and it has not been possible to obtain any evidence in support of the theory by means of microdissection. On the other hand, it has been possible to break some fibres into separate transverse discs.

Is the muscle substance attached to the sarcolemma?

The muscle substance can be made to separate or retract from the sarcolemma in a variety of ways. Mechanical trauma, heat, and, in fact, any of the numerous injuries which produce retraction clots (Speidel, 1939), cause groups of striations to run together leaving a clear, empty space within the sarcolemma. Agents causing rapid swelling, such as weak acids and alkalis, cause the muscle substance to herniate and flow out of the sarcolemma. Many contracture-producing drugs, or strong electrical stimulation, also tend to cause bulging of the sarcolemma (Ramsay & Street, 1940). The passage of a weak, constant current through a muscle fibre by non-polarizable micro-electrodes may also cause disruption of the muscle substance leaving an empty length of sarcolemma (unpublished observations). Microdissection has not so far revealed the presence of any intimate connexion between the sarcolemma and the underlying myofibrils. Such a connexion has frequently been postulated. Thus Häggquist (1931) suggested that the **Z** membrane is collagenous and extends right across the fibre to be attached to the sarcolemma. The fact that the sarcolemma can be separated from the muscle substance so easily and in so many ways, makes it rather unlikely that such a connexion exists. If there is any structural continuity it must be very weak and easily broken down. There is one special region in the muscle fibre at which a connexion between the myofibrils and the sarcolemma might occur, namely, the muscle-tendon junction. The exact mode of connexion between muscle and tendon in this region is a highly controversial subject. Some workers regard the myofibrils as being continuous with the tendon fibrils through small holes in the sarcolemma (Schultze, 1912). Others believe that the myofibrils end blindly in contact with the sarcolemma (Baldwin, 1913). For some reason spontaneous herniae caused by swelling are rather uncommon in the neighbourhood of the muscle-tendon junction. Such a hernia is, however, shown in Fig. 15. It will be seen that the muscle substance has poured out of the gap in the sarcolemma, leaving a clear space in the region of the muscle-tendon junction. Similar herniae have been produced artificially, and there seems to be no evidence of any special connexion of the myofibrils in this region to either the tendon fibrils or the sarcolemma. It is true that this evidence is based on fibres treated with acids and alkalis which might destroy such a connexion, but supporting evidence is given by Speidel (1939) who shows numerous drawings of retraction clots in the region of the muscle-tendon junction.

If the muscle substance is not connected to the sarcolemma either terminally or laterally, how is the force of the contraction transmitted to the connective tissue? I do not think it is necessary to postulate any intimate structural connexion between the muscle substance and the sarcolemma. We may very well imagine the relationship as similar to that between a sausage and its skin, or, to use another gastronomic analogy, the sarcolemma can be likened to the familiar sheet of cellophane paper used to cover jam. A long, thin cylinder of

highly viscous material surrounded by an elastic membrane could easily account for many of the properties of the muscle fibre. It would only be in the case of extreme contraction that we should expect the sarcolemma to bulge. Ramsay & Street (1940) have shown that the resting tension of single muscle fibres can be regarded as almost entirely due to the sarcolemma alone, and that applying a given pull to the sarcolemma gives the same stretch as that given by the intact fibre. This type of behaviour would be expected if the relationship between sarcolemma and muscle substance were a loose frictional one as suggested above, but would be more difficult to explain if the two were in structural continuity throughout their entire length. If it is admitted that the sarcolemma possesses a sufficient degree of elasticity, then there is no necessity to postulate any active relaxation mechanism (Kalckar, 1941; Ramsay, 1944), though, of course, this need not be excluded. Several of these features can be demonstrated in simple models consisting of thin elongated rubber sheaths containing a suitable material, such as a weak gelatin gel or thin putty. The rubber tends to cling to the contents yet is easily lifted up from them. A certain amount of bulging may occur at extreme shortening, and the elasticity of the sheath is sufficient to account for relaxation to the original length after shortening. If the relationship between contractile muscle substance and sarcolemma is a frictional or cohesive one, we might expect some degree of slip between the two surfaces at the initiation of contraction. Such an effect might account for part of the latent period between stimulation and the onset of development of tension. Sandow (1944), using a piezo-electric recording system almost free from inertia, has claimed that the muscle actually relaxes before the tension begins to rise. He has shown that there is probably a number of different processes going on during the latent period, and has suggested that myosin relaxes while it is being energized prior to contraction. It is possible that part of this latency relaxation may be due to stretching of the sarcolemma for a brief interval during which slip is occurring between it and the underlying muscle substance. In other words, the frictional equilibrium between muscle substance and sarcolemma in a muscle at rest is broken on initiation of the contractile process, and time is expended in setting up a new equilibrium.

The type of muscle model discussed here is not new. It was suggested by Gasser & Hill (1924) in an attempt to explain the viscous-elastic properties of muscle. They used models of thin rubber pipes filled with a very viscous material. Such models gave tension curves remarkably similar to muscles when subjected to quick stretches or releases. However, further work has shown that simple viscosity alone cannot account for the fact that active muscle shortens more slowly under a greater load; nor can it explain why extra heat is liberated when work is done (Fenn, 1923). To explain such effects it is necessary to make some assumption as to the manner in which the supply of energy to the contractile substance is regulated (Hill, 1938). However, such chemical considerations need not greatly disturb our general ideas on muscle structure, at least not so far as resting muscle is concerned. As Hill (1938) writes: 'The active

muscle is still a two-component system consisting of an undamped purely elastic element in series with a contractile element governed by the characteristic equation $(P+a)(v+b)=$ constant. There may be, indeed there must be, visco-elastic and probably purely viscous elements as well.'

It would, of course, be naïve to assume that the muscle substance behaves as a simple visco-elastic gel. There is a good deal of evidence which suggests that myosin undergoes a considerable change in physical and chemical properties during contraction (Deuticke, 1930; Mirsky, 1935). The close association, if not actual identity, of myosin and adenosine-triphosphatase is another factor which will probably have to be considered in attempting any explanation of the physical properties of the muscle substance.

Muscle swelling as a colloidal phenomenon

Colloidal swelling of proteins has been recognized for many years (see Lloyd & Shore, 1938). It is also well known that acids and alkalis are capable of increasing enormously the intake of water by protein gels. Most of the quantitative work has been done on gelatin, and the effect of hydrogen-ion concentration on the swelling of gelatin was first worked out theoretically and experimentally by Procter & Wilson (1916). According to their theory proteins can form ionizable salts with acids or alkalis at a *p*H remote from their iso-electric point. The presence of non-diffusible protein ions sets up a Donnan equilibrium, which results in an excess concentration of diffusible ions within the gel. This in turn causes an internal excess osmotic pressure, which results in swelling. The theory predicts a number of facts which are well borne out in practice. Thus the existence of an optimum *p*H for swelling, both in the acid and alkali range, is to be expected, and swelling should be minimal in the region of the isoelectric point. The presence of neutral salts should depress acid or alkali swelling by reducing the internal excess osmotic pressure (Lillie, 1907).

The swelling of muscle in acids and alkalis has been investigated by Loeb (1897) and Lloyd (1916). Both these investigators used whole muscles. No reference has been found to any similar work on single muscle fibres. In the case of whole muscles gain in weight has been taken as a criterion of swelling. This would be very difficult to determine in single fibres and would be unsatisfactory in any case. Indeed, it is scarcely possible to measure swelling in muscle quantitatively owing to the presence of the sarcolemma, the elastic resistance of which limits the amount of swelling of which the muscle substance is capable. Increase in diameter of the fibre is an unsatisfactory criterion owing to the escape of swollen muscle contents through herniation. Even for whole muscles gain in weight is a very unsatisfactory measure of swelling (or at least of the true maximum swelling of which the muscle substance is capable). If a whole muscle is immersed in an acid solution the degree of swelling of the individual fibres will depend on the amount and distribution of the connective tissue and on the position of the fibre. The surface fibres are least restricted and can swell most, whereas the central fibres are pressed upon by surrounding fibres and

connective tissue so that swelling will tend to be suppressed. In addition, the rate of penetration of the acid into the muscle may be an important factor, as, if penetration is slow, the outer fibres will be in contact with acid for a longer period than the inner fibres, which may in fact never become acid at all. It is probable that experiments on minced muscle or on muscle powder (Mirsky, 1936) would give a much truer quantitative picture of muscle swelling. Although it is not possible to investigate the swelling of single fibres in a satisfactory quantitative manner, a rough subjective estimate of the degree of swelling produced by various reagents can be made as previously described. Some of the results obtained with acids have already been discussed; and the existence of an optimum concentration for swelling has been noted.

The effects of alkalis on muscle fibres are very similar to those of acids. The muscle substance swells and both lateral and terminal herniation occur. Swelling is never so marked or so rapid as with acids. The effect of alkalis on the striations is variable. As a rule the striations remain visible at first and flow out, preserving their usual relationship. In most cases, however, the muscle after a few minutes tends to assume a glassy appearance, though some granular or fibrillar structure may remain. On the other hand, the structures are occasionally preserved indefinitely. The most striking difference between the effects of acids and alkalis is that the nuclei are never visible in the presence of alkali. As with acids, an optimum concentration for alkaline swelling exists. Thus in the case of potassium hydroxide the concentration range of 0·1–1 % seemed to be more effective than higher or lower concentrations. Similar effects were observed with other alkalis such as sodium carbonate and tribasic sodium phosphate.

In accordance with the Proctor-Wilson theory salts were found to exert a strong depressant action on both acid and alkali swelling. Acids made up in physiological saline or Ringer's solution were much less effective as swelling agents. In sharp contrast to this, non-electrolytes, such as urea, glycerol and glucose, appeared to exert no action. Even saturated solutions of urea did not affect the action of acids. These results are in accordance with those of Lillie (1907), who found that the osmotic pressure of colloids is depressed by salts but unaffected by non-electrolytes.

An attempt was made to determine the *p*H at optimum swelling by means of buffers. One of the difficulties in this procedure is that, as we have seen, salts depress acid and alkali swelling. The Michaelis acetate-veronal buffers having a constant ionic strength from *p*H 2·62 to 9·64 were used (Michaelis, 1931). These gave a reasonable degree of swelling in the acid and alkaline range. The acid maximum occurred at about *p*H 3 and swelling was almost negligible at *p*H 4·3. From *p*H 4·7 to 8·5 no swelling occurred, but there was a rapid increase in swelling above this *p*H level. The alkaline optimum could not be determined by buffers alone, but the use of unbuffered alkaline solutions suggests that it is located at about *p*H 10.

Comparison with whole muscle and myosin. The swelling curves for whole

muscle, based on change in weight, have been described by Lloyd (Lloyd, 1916; Lloyd & Phillips, 1933; Lloyd & Shore, 1938). These curves show sharp maxima at *p*H 2·2 and 11·8 and a marked stability zone from *p*H 5·0 to 8·0. These results are in good agreement with those given above, using single fibres. Lloyd (1916) found that the maximum swelling with hydrochloric acid occurred at a concentration of 0·005 *N* (0·018 %), in excellent agreement with my own figure of 0·02 %. There is some discrepancy in the alkaline range, as Lloyd obtained maximum swelling in 0·005 *N* caustic soda. This concentration produces very little visible effect on single fibres, and maximum swelling occurs in solutions from ten to one hundred times more concentrated. The discrepancy seems large when expressed in terms of concentrations of alkali, but corresponds to only one or two units when expressed in terms of *p*H. Bearing in mind the differences in material and method the general agreement in behaviour between whole muscle and single fibres is reasonably close.

Lloyd has drawn attention to the fact that structural organization of a protein may exert a profound influence on swelling. As the closeness of texture of the structure increases, the maxima of acid and alkaline swelling become less pronounced, and the zone of minimal swelling becomes more extended. Thus gelatin shows two sharp maxima, with a fairly sharp minimum at *p*H 5. Collagen, however, shows a zone of minimum swelling extending from *p*H 4 to 8·5. Unfortunately, very few data exist regarding the swelling of the main muscle protein, myosin. Weber (1925) investigated the swelling of myosin gels in the acid region. He found a fairly sharp minimum at *p*H 5·2 which he considered to be the isoelectric point of myosin. Swelling increased very rapidly between *p*H 5·2 and 4, but only very slowly between *p*H 5·2 and 7.

In the case of myosin fibres, Weber (1934) found minimal swelling between *p*H 5 and 5·5, with a slow increase in swelling between *p*H 5·5 and 9. A certain amount of indirect information can be obtained from examination of the acid-base titration curve of myosin, since in general the swelling curve of a protein shows certain similarities to the titration curve. Dubuisson (1941) has carried out a careful investigation of the titration curve of myosin. His results show that myosin has a fairly sharp isoelectric point at *p*H 5·45. The curve then rises fairly steeply on the acid side of this, but is relatively flat on the alkaline side up to *p*H 8 or even 9.

This type of behaviour is roughly in accordance with what we might expect from studies on other proteins and fibrous structures. Whole muscle has a stability zone from about *p*H 5 to 8. Single fibres show no gross swelling between *p*H 4·7 and 8·5. Myosin fibres show a sharper minimum between *p*H 5 and 5·5, with a relatively flat zone up to *p*H 9, and the indications are that myosin gels show a sharp minimum at about *p*H 5·2. As suggested by Lloyd an increase in structural organization of the protein leads to an increase in the width of the stability zone.

As noted above, organic acids appear to be slightly more active as swelling agents than the mineral acids. A similar effect was noticed by Speakman &

Hirst (1933) who investigated the swelling of wool fibres. They found, for example, that formic acid was twice as effective as sulphuric acid in facilitating fibre extension, and many times as effective in producing swelling. The reason they suggested for this was that wool forms highly ionizable salts with weak acids, which tend to depress the ionization of the free acid within the fibre. This causes an increase in the excess of diffusible ions within the fibre, and hence an increase in swelling. On this theory the swelling of the fibre at any given *p*H should be inversely related to the ionization constant of the acid used. It seems probable that similar considerations will apply in the case of muscle fibres.

Swelling of fixed and frozen-dried muscle fibres

A number of experiments were carried out on reconstituted frozen-dried muscles. The muscles were frozen rapidly in isopentane at $-170°$ C. followed by drying *in vacuo* at $-40°$ C. On being allowed to soak in Ringer's solution water was taken up and the tissue resembled fresh muscle in all respects except that it was no longer contractile. Single fibres were teased out and subjected to the action of various reagents. So far as swelling under the influence of acids or alkalis is concerned such fibres behave in very much the same way as fresh fibres. Thus none of the structures which produce the characteristic changes following application of weak acids or alkalis to fresh fibres appears to be affected by the freezing-drying process.

With regard to fixatives the results seem to depend on the efficiency of the fixation. Thus after prolonged formalin fixation weak acids produced no gross swelling, though the nuclei became visible. With 96 % alcohol, on the other hand, swelling and herniation can occur even after prolonged immersion (Figs. 16, 17).

The muscle nuclei

In a fresh untreated fibre the muscle nuclei are normally invisible. Within a few moments of addition of a weak acid many hundreds of nuclei appear. If herniation occurs the nuclei flow out with the muscle substance. The arrangement of nuclei differs in different species and in different types of muscle. It is generally stated that in mammals the nuclei are peripheral and lie immediately beneath the sarcolemma. In frogs they are said to be scattered throughout the muscle substance. In general, these statements have been confirmed by microdissection. The exact relationship of the nuclei to the muscle substance is difficult to determine in the frog, but in the white muscle fibres of the rabbit the nuclei can often be seen resting on, but not embedded in, the herniated muscle substance (Fig. 13). It is occasionally possible to pull off a nucleus from the surface of the muscle substance, but more often one or more myofibrils are torn at the same time. Thus it is uncertain how closely the nuclei are attached to the myofibrils. They may be embedded in the sarcoplasm or in actual anatomical continuity with the surfaces of the myofibrils. In the frog one almost gains the impression that there is one nucleus attached to each myofibril. This would be difficult to prove without detailed measurements and

counts, but simple calculations show that the hypothesis is by no means untenable, even in the case of mammalian fibres, in which the nuclei are usually stated to be mainly peripheral. Purely geometrical considerations show that assuming one nucleus is attached to each myofibril, the probability of seeing a nucleus in any random cross-section is greatest at the periphery of the fibre and decreases progressively to almost zero at the centre of the fibre. It can be shown that a small muscle fibre containing large myofibrils may give the impression that most of the nuclei are peripheral, whereas a more even distribution of nuclei may be suggested in a large fibre containing small myofibrils. It is clear that more knowledge of the size of myofibrils in different species as well as detailed nuclear counts will be required before we can assume any special distribution of nuclei. At present the muscle fibre is usually regarded as a syncytium, but it is well to bear in mind the possibility that it may be composed of a number of individual cells each with its own nucleus. It must be admitted that such an hypothesis is very difficult to reconcile with the conditions found in certain muscles. Thus in many insect muscles the nuclei undoubtedly occur as a central core, and in some red muscles of rabbits they appear as a number of long regular columns running along the fibres.

Shape. In mammals the nuclei are usually oval in shape. In Amphibia there is considerable variation in form, many being oval but some being very elongated and tortuous (Fig. 4). Within the fibre the nuclei are usually flattened and elongated, but in the herniated muscle substance they frequently become more rounded and swollen, as if they had been released from a constraint (Fig. 17). It is not possible to say whether such a constraint exists in the normal fibre or whether it is the result of swelling.

The reversible appearance and disappearance of nuclei

If a muscle fibre is treated with dilute acids it swells and the nuclei becomes visible. If a fibre is treated with dilute alkalis it also swells, but the nuclei remain invisible. The question now arises, can nuclei which have been made visible by the action of acids be made to disappear by treatment of the same fibre with alkalis? The answer is that they can, and, moreover, the same nuclei can be made to reappear by the further application of acids. Indeed, the process seems to be completely reversible and can be repeated indefinitely. Fig. 19 shows a low-power view of a fibre in which the nuclei have been made visible by dilute acid. Fig. 20 shows the same fibre subjected to alkali. The nuclei have disappeared. In Fig. 21 the nuclei have reappeared following further treatment with acid. This series of photographs was taken after the nuclei had been made to appear and disappear ten times. Figs. 22 and 23 show a high-power view of the same fibre illustrating the same effect for the eleventh time. The remarkable thing about this effect is its reversibility. It has been repeated as many as fifty times in the same fibre, and could no doubt be repeated almost indefinitely. What happens to the nuclei when they become invisible? Attempts to answer this question were made using dark-ground illumination and dyes.

If a muscle fibre is examined under dark-ground illumination while being treated with dilute acids, the nuclei begin to appear as shining specks of light which gradually become larger. If herniation occurs the appearance of hundreds of bright nuclei flowing along against a dark background is very spectacular. If the fibre is now treated with dilute alkali the bright nuclei gradually fade and eventually disappear entirely. The whole process can best be observed by subjecting the fibre to ammonia fumes, thus avoiding any movement of the specimen and enabling any single nucleus to be observed without disturbance. Thus dark-ground illumination gives no answer to the problem, beyond showing that acids cause the appearance of granular structures which can be shown up in the dark field. The use of dyes led to some unexpected results. If an acid-treated fibre is treated with a solution of a basic dye the nuclei become stained within a few seconds. The concentration of the dye does not matter much, but excess dye should be washed off in saline in order to prevent staining of the cytoplasm. If the fibre is now treated with dilute alkali the nuclei begin to fade and may disappear altogether. As a rule the nucleoli are the last structures to fade. With many dyes the nuclei become completely invisible and no trace of colour remains. If such nuclei are then acidified once more they reappear, and, moreover, they appear stained in the original colour. The reason for this curious effect is partly that many of the commonly used basic dyes, such as methyl green, thionine, and gentian violet, are acid-base indicators and change colour in alkaline solution. This cannot be the whole story, however, since the nuclei often disappear at *p*H's lower than those at which the dyes change colour. However, in order to eliminate any such effect, methylene blue, which is scarcely affected by alkali, was used. With this dye it is rarely possible to make the nuclei disappear altogether. They are usually just visible as a very faint light bluish haze. No structure can be made out. If the fibre is now acidified the nuclei become granular or reticular and these structures take on a deep blue colour. This type of behaviour suggests that the nucleoproteins in the nuclei are coagulated or precipitated by acids and dispersed or dissolved in presence of alkalis. This fits in with the known properties of nucleoproteins, which tend to precipitate at their isoelectric points, which usually lie between *p*H 3 and 5. Using buffers it was found that the nuclei of frog muscle become visible at about *p*H 4·3. This is in good agreement with the observations of W. H. & M. R. Lewis (1924), who found that the nuclei of cells in tissue cultures became visible at *p*H 4·6–3·8. The process was reversible to some extent, but the cells did not live so long as untreated cells. Kuwada & Sakamura (1927) investigated the behaviour of the chromosomes of the pollen mother cells of *Tradescantia virginica*. They found the chromosomes were clearly visible between *p*H 2·6 and 5·2, but became indistinct above this, being quite invisible at *p*H 6·6. Experiments with neutral violet extra showed that good staining of the chromosomes was obtained only below *p*H 4·38. Unfortunately, no attempt seems to have been made to see whether the stained chromosomes could be made to appear or disappear by alteration of the *p*H.

The chief interest of the present observations lies in the demonstration of the remarkable degree of reversibility of the effect and in showing that the reaction between dye and nucleus can be altered reversibly in the same nucleus. It would be interesting to know more about the structure of the nucleus in the 'invisible' state. Phase contrast or ultra-violet microscopy might be helpful here. It would also be important to know whether the form of the nucleus is exactly the same after it has been made to disappear and reappear a number of times. Preliminary observations on the giant salivary gland chromosomes of *Chironomus* larvae suggest that this is in fact the case.

DISCUSSION

Early observations on the effects of acids on muscle. It is curious that although many workers, especially during the latter half of the last century, have examined muscles treated with acids, practically no reference is made to the striking phenomenon of rupture of the sarcolemma accompanied by flow of striations. There are several possible reasons for this. In many cases insect muscles were studied, and unjustifiable conclusions were drawn as to the structure of vertebrate muscles. Insect muscles swell in acids, but the phenomenon is not nearly as striking as in vertebrates. I have not as yet observed lateral herniation in insect muscle, and certain insect wing muscles do not appear to possess a sarcolemma. To observe the phenomenon at its best requires a number of conditions, such as the use of unfixed muscle, a fairly narrow range of acid concentration, and the absence of salts. The desire to study muscle structure under high power may have caused some workers to miss the phenomenon, which is very haphazard and may only affect one part of a fibre or may even not occur at all in the fibre under observation. Those few observers who do comment on the effect do not appear to have watched the actual flow of striations but merely saw it as a static effect. Thus Ranvier (1880) remarks that following application of dilute acids 'la substance musculaire se gonfle et fait hernie comme une sorte de champignon à l'extrémité des fibres rompues. Au riveau de l'union de cette excroissance avec la continuité du faisceau primitif, le sarcolemme se rétracte et forme des plies transversaux comparables à ceux de la manche retroussée d'un vêtement.' No reference is made to lateral herniae. In recent times Nagel (1935) shows a photograph of a lateral hernia produced artificially by means of a microneedle, and suggests that this might be a useful method for studying the properties and structure of the sarcolemma. He makes no reference to spontaneous herniation. This paucity of description is all the more remarkable in that an excellent discussion of these herniae and their relation to the sarcolemma was given by William Bowman (1840) in his celebrated paper 'On the minute structure and movements of voluntary muscle'. This contains several beautiful drawings of herniae, one of which (Fig. 18) is reproduced for comparison with Fig. 17. Bowman writes: 'The sheath dilates at first but soon grows tense; the effect of which on the fibrillae is that they emerge at its open extremity, where the fasciculus has been broken

off, and there swell in diameter; or, if their union is but slight, expand in all directions into a roundish mass or button. Occasionally, in thus emerging they curl back upon itself the rim of the sarcolemma which is then seen to grasp them still more tightly. But if this vent be insufficient, and especially if the fragment be long, so that its ends cannot be reached soon enough, the over-distended membrane gives way, bursting at detached points and allowing the escape of the contents.'

Does muscle swelling occur in the living body? Most of this work on muscle swelling has been carried out using lactic acid. Now it is well known that lactic acid is liberated during muscular work. Fletcher & Hopkins (1907) showed that resting muscle contains 0·02 % lactic acid, working muscle may contain 0·2 %, and fatigued muscle may contain up to 0·4 %. Meyerhof & Lohmann (1926) found concentrations of lactic acid up to 0·57 % in extreme fatigue. Now lactic acid is a very good swelling agent in this range of concentrations; indeed, swelling of single muscle fibres can be observed in concentrations as low as 0·005 %. True, swelling is depressed very considerably in the presence of saline or Ringer's solution, but even so we should expect considerable swelling with concentrations of acid above 0·4 %. A. V. Hill and his co-workers have discussed the fate of lactic acid liberated during muscular work (Hill, Long & Lupton, 1924; Hartree & Hill, 1923). Enormous quantities of lactic acid, 50 g. or more, may be liberated in 30 sec. of severe exercise, and blood concentrations of over 100 mg./100 c.c. may be attained. The local concentration in the muscles is probably, for a short time at least, much higher. The *p*H of both blood and muscle must fall in severe exercise, but too great a fall is prevented by the buffering power of the tissues and blood. According to Dubuisson (1941) the buffering capacity of myosin is such that it is only capable of neutralizing about 15 % of the lactic acid formed during fatigue, and even the combined action of myosin and myogen can only account for 30 % of the acid. Even allowing for the presence of some other protein of high buffering capacity the possibility exists that some muscle fibres may be exposed to rather high local concentrations of lactic acid during severe exercise. The concentration may not be uniform throughout the muscle but may depend on circulatory factors. Thus it is possible that the blood supply to a certain group of fibres may become deficient during severe and sustained exercise so that lactic acid may be produced under anaerobic conditions, and this lactic acid would be temporarily unable to escape into the general circulation. In such circumstances we might reasonably expect swelling and even herniation of fibres. There is, in fact, good evidence that swelling can occur in active muscles. Fletcher (1904) found that excised fatigued muscles imbibed more water than resting muscles. Barcroft & Kato (1915) showed that mammalian muscles stimulated to fatigue *in situ* increased in weight, and Back, Cogan & Towers (1915) found the same in frog muscles. Unfortunately, none of these workers examined their material histologically. We are still ignorant as to the causes of many of the symptoms, such as pain and stiffness, which follow violent muscular exercise. It is reasonable to

believe that stiffness may be related, in part at least, to muscle swelling. Pain is more difficult to explain. A few scattered herniae in a muscle might very well account for certain types of muscle pain following exercise. It is well known that proteinuria may follow severe muscular exercise (Covian & Rehberg, 1936). The evidence concerning the effect of exercise on protein metabolism is rather conflicting, but Cathcart (1925) came to the definite conclusion that there is increased protein catabolism after exercise. Is it not possible that part of this increased protein breakdown and temporary proteinuria may be due to disruption of muscle fibres by swelling in very severe exercise? An attempt to answer this question is now in progress.

The physiological effects of acids on muscles. A number of workers have investigated the effects of acids on such properties as the excitability and force of contraction of muscles. Thus Dale & Mines (1911) recorded muscular contractions in 0·01 *N* acids and in various acids at *p*H 2. Lapicque & Nattan-Larrier (1926) measured the chronaxie of muscles immersed for several hours in baths at various *p*H's down to *p*H 2. Recently, Finnerty & Gesell (1945) compared the action of acids on the respiratory and locomotor muscles, using a *p*H range down to 3·1. In no case does there appear to have been any check on the structure of the muscle. If a muscle is immersed for only a few minutes in acid solutions at about *p*H 3, it is found that the outermost fibres are grossly swollen and sometimes herniated. Such fibres are certainly abnormal and probably inexcitable. Single muscle fibres at *p*H 3 do not respond to galvanic stimuli applied through microelectrodes. It is possible that they might respond to much stronger stimuli or to different types of current, but this seems unlikely. It is very probable that what these workers were dealing with was an inner core of surviving muscle fibres surrounded by a layer of dead or inexcitable fibres. The longer the muscle is kept in the acid bath the fewer the surviving fibres. It is likely that many unjustifiable conclusions could be avoided if an adequate 'anatomical control' were carried out. Many physical and pharmacological agents alter cell structure profoundly, and it is important to know the anatomical state of the tissue in an experiment as well as its physiological properties.

The structure of the muscle fibre. It is too early to attempt any detailed picture of muscle structure. However, it may be useful to discuss some of the properties of the muscle fibre, particularly in relation to recent work with the electron microscope. Using this instrument, Hall, Jakus & Schmitt (1946) have been able to take very highly magnified photographs of myofibrils which apparently settle, once and for all, many controversial points. In the first place the actual myosin fibrils are visible and run without interruption through both the anisotropic (*A*) and isotropic (*I*) discs. Even in very contracted myofibrils the myosin threads remain straight and do not appear to be folded or arranged in a spiral formation. Many attempts have been made to explain why the *A* disc is strongly birefringent while the *I* disc is not. At first it was suggested that all the myosin was concentrated in the *A* disc. More exact analyses of the

myosin content of muscles (see Bailey, 1944) makes this suggestion unlikely. Bernal (1937) put forward the interesting suggestion that the myosin chains are arranged in a reversed spiral formation, leading to a more or less parallel packing of the myosin molecules in the *A* discs. The electron microscope pictures afford no support for such a theory, at least not on the level of organization rendered visible by present magnifications. It is true that in the *A* discs the myosin threads appear to be denser and more parallel than in the *I* discs. Schmidt (1934) has shown that the *I* discs are in fact faintly birefringent, but the difference in birefringence between the *A* and the *I* discs is probably too great to be accounted for by such slight differences in orientation alone. It seems likely that we must postulate the presence of substances in the discs which either mask or enhance the birefringence of myosin. Caspersson & Thorell (1942) have claimed that the adenine derivatives present in muscle are localized in the *I* bands of resting muscle. It is interesting to note that Needham and his co-workers (Dainty *et al.* 1943) found that adenosine triphosphate caused a decrease in birefringence of flow of myosin solutions. We may perhaps speculate as to whether it can mask the birefringence of myosin in the *I* discs. Micro-incineration work (Scott, 1932) has shown that most of the mineral ash is localized in the *A* bands. The effect of various mixtures of salts on the birefringence of myosin is not known, but it is fair to point out that potassium salts decrease the flow birefringence of myosin solutions.

Whatever may be the explanation of the difference in birefringence between *A* and *I* discs, two things are clear. First, the myosin fibrils run along the entire length of the muscle fibre, with only a slight difference in orientation in the *A* and *I* discs. Secondly, there is a regular periodic distribution of salts and other substances along the length of the fibre. The inference is that any differences in properties of the *A* and *I* discs are due not to differences in arrangement of the myosin molecules, but to the presence of different chemical substances in contact with the myosin. If most of the salts are in fact restricted to the *A* bands then the myosin in those bands must be in a very different state from that in the *I* bands. Furthermore, it is impossible to believe that the physical and chemical properties of myosin, which is either identical or closely associated with adenosine triphosphatase, are not profoundly affected by the presence of adenosine triphosphate in the *I* bands alone. Indeed, it is rather surprising that the differences in orientation of the myosin in the two bands are not greater than they appear in the electron microscope photographs. We are not in a position to explain why different chemical substances are localized in different regions of the fibre. There is no evidence of any definite membrane between the *A* and *I* bands. For the present we must accept this periodic distribution as a fact, and we must also expect a periodic variation in such things as the ionic atmosphere and distribution of electrical charges along the fibre. Such a concept may be of importance when trying to explain certain puzzling microscopic features of muscle structure. As we have seen muscle fibres can be split up longitudinally into striated myofibrils or groups of striated myofibrils. Now as

a rule in a whole fibre the striations are seen to extend right across the fibre. This means that the striations of the individual myofibrils must be in perfect alignment. When, as occasionally happens, they are not, they may give rise to a 'vernier' effect or even a spiral appearance. On the other hand, as was first recognized by Bowman (1840), after treatment with certain reagents the fibres can sometimes be broken down into flat discs. The existence of these discs has been doubted by some, but they can quite often be demonstrated by micro-dissection and are sometimes very evident in fixed and stained degenerating fibres. A very good example of fragmentation into discs is shown by Le Gros Clark (1946, Text-fig. 2), and another photograph taken from his material is reproduced in Fig. 24. This shows evidence of zigzag splitting into discs and groups of myofibrils, and should be compared with Text-fig. 1. The most obvious explanation for these effects is that myofibrils do exist, but that some sort of membrane stretches right across the fibre, through the sarcoplasm and unites the myofibrils laterally. From examination of fixed and stained material it has been suggested that the *Z* membrane fulfils these conditions. Some authors regard the *Z* membrane as collagenous and attached to the sarcolemma (Peterfi, 1913). Some of the evidence against the latter view has already been discussed. Electron microscopy shows that although the substance of the *Z* membrane does appear to cement the myosin threads together within the myofibril, there is no evidence of its being collagenous in nature. Also the peripheries of the *Z* discs are quite sharp and show no evidence of having been torn, as might be expected if they extended between adjacent myofibrils. Further evidence against this view comes from observation of contractions of individual myofibrils or groups of myofibrils (unpublished work). If the myofibrils were all interconnected contraction of individual myofibrils would be extremely difficult. Thus we must find some alternative explanation for the alignment of the myofibrils and their cleavage into discs under certain circumstances. In recent years considerable progress has been made towards understanding the nature of the long-range forces which exist between colloidal particles (Langmuir, 1938; Bernal & Fankuchen, 1941; London, 1942; Levine, 1946; Verwey & Overbeek, 1946). Such long-range forces are in all probability exceedingly important in biology (Szent-Gyorgyi, 1941), and it is possible that they may be responsible for the alignment of myofibrils. Unfortunately, the theoretical basis for these forces is in dispute, and it is not yet possible to say how they originate. Nevertheless, it is certain that they must exist and may act over very considerable distances—even up to $0{\cdot}5\mu$. London (1942) has suggested on theoretical grounds that centres of attractive force may exist at rather wide intervals along chain-like molecules. He has used such centres of attraction to explain the elasticity of rubber. We have already seen that there is a periodic distribution of chemical substances along the muscle fibre; is it not possible that this may bring about a periodic distribution of attractive forces? Thus at certain regions of the myofibril there may be strong forces binding the myosin threads together. At the margin of the myofibril these

forces are incompletely satisfied and residual forces exist which tend to attract similar regions in neighbouring myofibrils. In such a manner we could perhaps explain the alignment of striations without invoking the existence of actual membranes. The formation of discs would not be difficult to understand on this basis. Under the influence of certain chemicals such as acids or formalin the lateral attractive forces might become greatly exaggerated so that the cross striation becomes accentuated and fragmentation into discs may occur.

SUMMARY

1. The effects of weak acids on single muscle fibres are described. In suitable concentrations of dilute acids (e.g. 0·2% lactic acid) the fibres swell and spontaneous ruptures of the sarcolemma occur, with herniation of the muscle substance. At the same time the nuclei become visible.

2. Microdissection of the herniated muscle substance shows that the latter can be separated easily into groups of striated myofibrils, but occasionally transverse discs can be dissected out.

3. Observation of the cross-striations during swelling suggests that they behave as viscous deformable gels.

4. The relationship between the muscle substance and the sarcolemma is considered. Many of the properties of the muscle fibre can be exhibited in models composed of a thin elastic rubber membrane containing a viscous semi-fluid material.

5. Muscle swelling is considered in relation to theories of the colloidal swelling of proteins, and the swelling properties of myosin, myosin fibres, single muscle fibres, and whole muscles are compared.

6. The action of acids in rendering nuclei visible is reversed by alkali. Nuclei can be made to appear or disappear at will by alteration of the *p*H.

7. These results are discussed in relation to muscular fatigue. Possible errors in previous work on the physiological action of acids on muscle are suggested. The importance of an 'anatomical control' in physiological experiments is stressed.

8. Muscle structure is discussed in relation to recent work with the electron microscope. It is suggested that the properties of different regions of the fibril depend not so much on different arrangements of the myosin in those regions as on modifications of the physical and chemical properties of the myosin due to the periodic distribution of various chemical substances, particularly salts and adenosine triphosphate. Such periodic variations may in turn cause a periodic distribution of long-range forces along the fibril. These forces may be responsible for the alignment of myofibrils, and in the presence of certain substances may become so strong that the fibre is readily broken into discs.

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APPENDIX

A SIMPLE MICROMANIPULATOR

By E. H. J. SCHUSTER

(Figs. 25, 26)

The needle or pipette is held in a fitting (not shown) clamped to the spindle (1) at its left-hand end, where its diameter is reduced. This spindle can slide in the sleeve (2) and its position is determined by the compression spring (3) which tends to force it to the right by its action on the end-plate, and the push rod (6) by which it may be moved to the left. The push rod is carried in the sleeve (7), and motion may be imparted to it by rotation of the long cap-nut (8). The left-hand end of the rod is rounded, hardened and polished and bears on a hardened and polished steel plate (5) held in the member (4) by a spigot and nut. The member (4) is bored to hold the spindle (1) to which it is rigidly fixed. Thus the forward movement of the needle holder is given by rotation of the cap-nut (8) about its axis. The lateral and up-and-down movements are brought about by movements of the sleeve (7) in the reversed directions, for the sleeve acts as a lever of the first order, its fulcrum being the ball and cone shown in the member (9). The motion is imparted to the needle holders through the second (left-hand) steel ball which is a close fit in the steel bush mounted in the member (10). Member (10) is held hard up against member (9) by bolt and compression spring and washer (11). Lateral movement of the lower end of (10) is prevented by the pin (12) carried on (9) and working in a slot in (10). Thus when cap-nut (8) is depressed member (10) rises in a proportion of about 1 to 20 carrying with it the sleeve (2) and the spindle (1); and when the cap-nut is moved laterally the upper end of (10) moves along a circular arc whose centre is at (12). The function of the pin (13) which passes through a hole in member (4) is to prevent rotation of the spindle. The parts hitherto described may be mounted in a variety of ways; in the diagram they are represented as being clamped to the vertical member (14) of the stand by a bolt and coning nut. Member (9) fits a groove in (14) and the bolt which is fixed in (9) traverses a slot running along the centre of the groove. This allows for vertical adjustment of the working parts of the apparatus.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Frog muscle fibre treated with 0·2% lactic acid, showing a spontaneous lateral hernia. ×150.

Fig. 2. Frog muscle fibre treated with 0·2% lactic acid, showing a spontaneous lateral hernia through a large circular rupture of the sarcolemma. ×150.

Fig. 3. Frog muscle fibre treated with 0·2% lactic acid, showing spontaneous terminal hernia. Note sharp rim of sarcolemma. ×150.

Fig. 4. Frog muscle fibre treated with 0·2% lactic acid, showing terminal hernia in which the entire muscle contents have become extruded leaving an empty sarcolemmal sheath. Note the curled-up retracted rim of the sarcolemma, and the tortuous muscle nuclei. ×150.

Fig. 5. Frog muscle fibre treated with 0·2% lactic acid, showing a lateral and a terminal hernia in the same fibre. The sharp circular rupture of the sarcolemma is seen in the lateral hernia. ×150.

Fig. 6. Rabbit muscle fibre treated with 0·2% lactic acid, showing a series of multiple spontaneous ruptures in the sarcolemma with bulging of the swollen muscle substance. A similar appearance is often seen in swollen collagen or cotton fibres. ×200.

Fig. 7. Frog muscle fibre treated with 0·15% lactic acid, showing two lateral herniae produced artificially by means of a microneedle. Such herniae are usually very small. The larger of the two has been enlarged by micromanipulation. ×200.

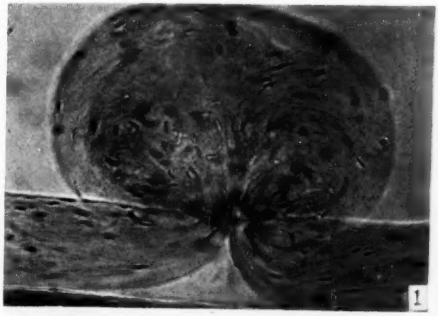
Fig. 8. Rabbit muscle fibre treated with 0·15% lactic acid, showing a long loop of muscle substance which has herniated spontaneously through a rupture in the sarcolemma (not shown). Note the preservation of the striations. ×200.

Plate 2

Fig. 9. Rabbit muscle fibre treated with 0·15% lactic acid, showing a herniated loop which is being stretched between two microneedles (outside the field of view). ×600.

Fig. 10. The same fibre as in Fig. 9, on release of tension. The loop has become rounded, making focusing difficult, but the striations are much closer together. ×600.

Fig. 11. Rabbit muscle fibre treated with 0·2% lactic acid, showing a loop being stretched between two microneedles, in a direction transverse to the fibre axis. A number of bands of striated myofibrils are seen. ×200.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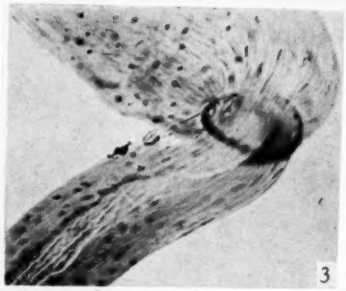
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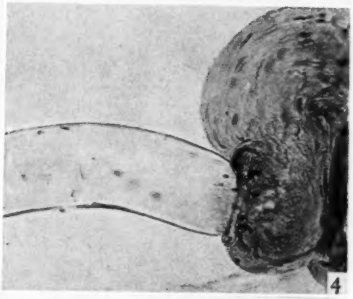
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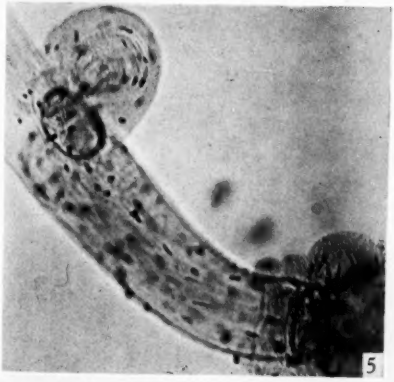
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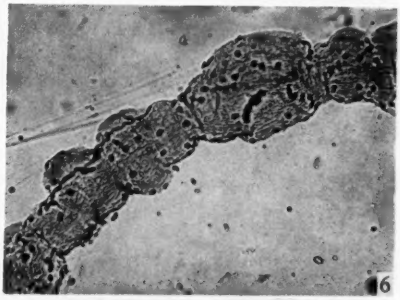
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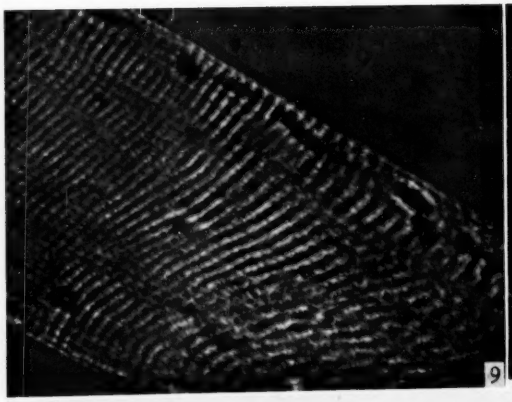
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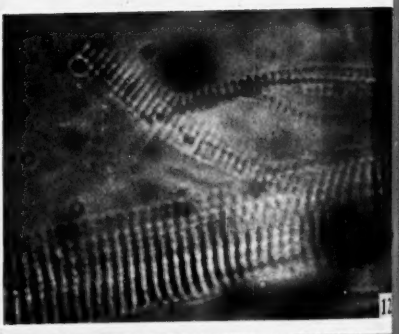
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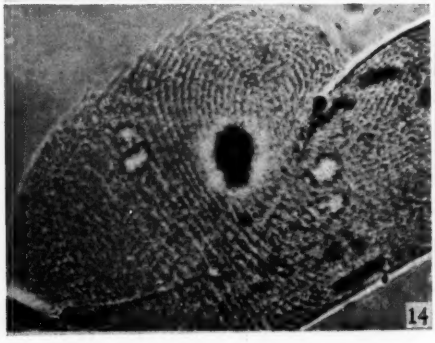
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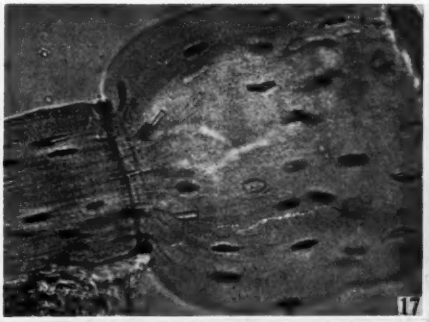
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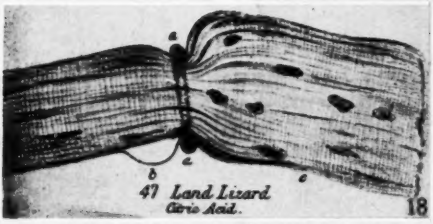
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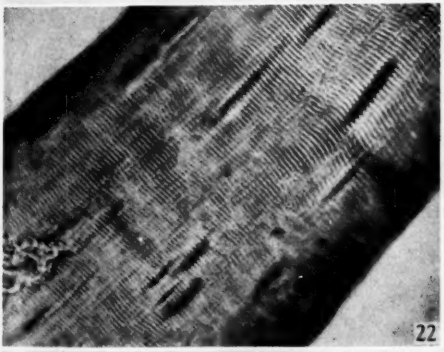
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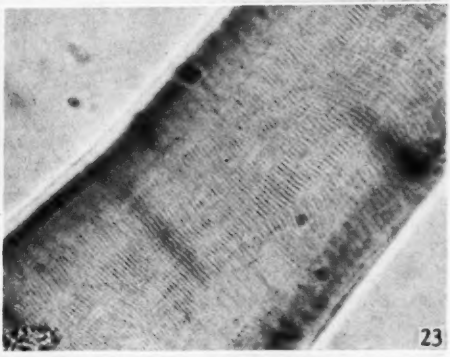
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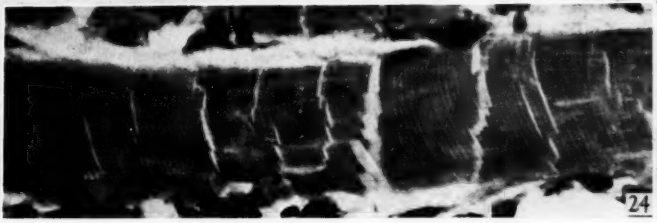
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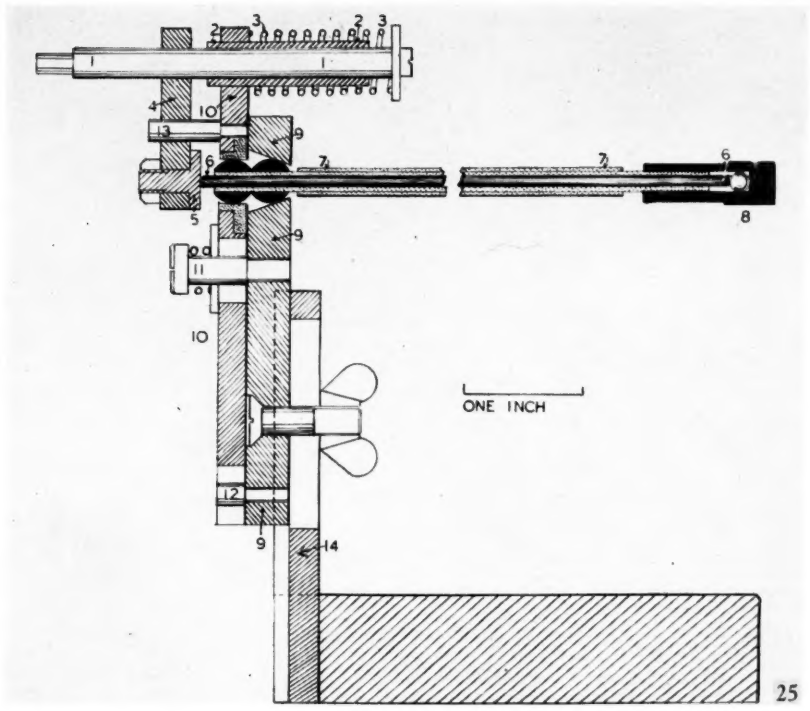
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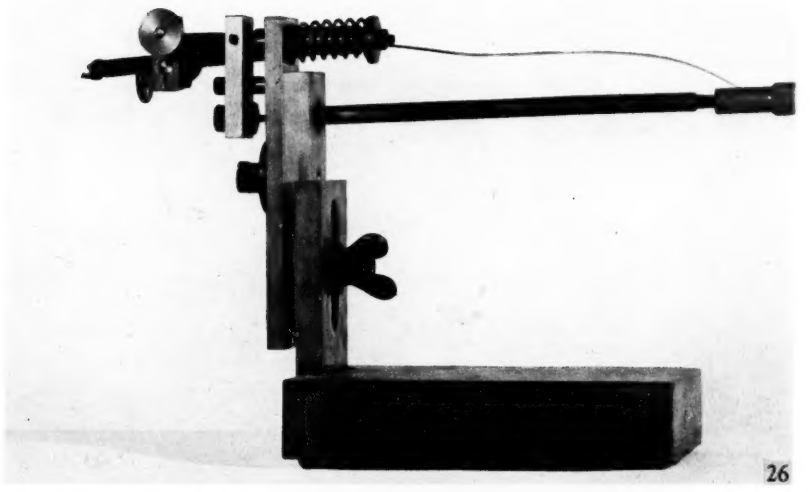
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Fig. 12. High-power view of a group of myofibrils from the same fibre. Even the thinnest bands are striated. ×800.

Fig. 13. Rabbit muscle fibre treated with 0·2% lactic acid, showing 'finger-print' distortion of striations in a lateral hernia. One of the nuclei is seen to be resting on the surface of the muscle substance, not embedded within it. ×200.

Fig. 14. Rabbit muscle fibre treated with 0·2% lactic acid, showing a lateral hernia through which the striations have flowed, increasing in length and becoming curved at the edges. ×600.

Fig. 15. Frog muscle fibre treated with 0·2% lactic acid, showing a spontaneous lateral hernia near the muscle-tendon junction. The muscle substance has retracted from the muscle-tendon junction leaving a clear space within the sarcolemma. ×150.

PLATE 3

Fig. 16. Frog muscle fibres treated with 0·2% lactic acid after fixation for 24 hr. in 96% alcohol. Spontaneous lateral herniae. ×150.

Fig. 17. Frog muscle fibre treated with 0·2% lactic acid after fixation for 7 days in 96% alcohol. Spontaneous terminal hernia. Some of the nuclei in the herniated region tend to be more rounded than those still within the sarcolemma. ×600.

Fig. 18. Photograph of one of Bowman's drawings for comparison with Fig. 17.

Fig. 19. Frog muscle fibre treated with 0·2% lactic acid. The nuclei have become visible. ×200.

Fig. 20. Same fibre subjected to ammonia vapour for a few seconds. The nuclei have disappeared.

Fig. 21. Same fibre re-acidified with 0·2% lactic acid. The nuclei have reappeared.

Fig. 22. High-power view of part of Fig. 21. ×600.

Fig. 23. Same fibre subjected once more to ammonia vapour. The nuclei have disappeared and the fibre has shrunk. ×600.

Fig. 24. Dead muscle fibre from a gracilis graft in a rabbit. Under the action of the fixative the fibre has split in a series of steps. Compare with Text-fig. 1. Fixed in formalin. Stained with haematoxylin and eosin.

PLATE 4

Fig. 25. Diagram showing sectional elevation of micromanipulator.

Fig. 26. Photograph showing micromanipulator with micro-electrode in holder.

VARIATIONS IN THE DISTRIBUTION OF LIPOIDS IN THE ADRENAL CORTEX OF THE ALBINO RAT

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It is exceedingly difficult to distinguish between the different lipoids present in the adrenal cortex. Bennett (1940) appears to have been the first to apply to it a wide variety of histochemical tests. Gottschau (1883) and most subsequent workers (see Baxter (1946) for summary and additional evidence) are in agreement that cells are proliferated in the region between the z. glomerulosa and z. fasciculata and move inwards towards the medulla. Bennett (1940), working with the adrenals of the cat, has described four zones in the cortex—pre-secretory, secretory, post-secretory and senescent, from without inwards—the last being not always present. These zones vary with age and sex and after castration. The adrenal cortical sterones he found to be confined to the secretory zone in both sexes and at all ages. Deane & Greep (1946) have recently produced evidence from morphological and histochemical methods that, in the rat, the cells of the z. fasciculata arise in the 'subglomerulosal' layer and move inward to die in the z. reticularis; the cells of the z. glomerulosa, however, do not migrate.

The present paper describes the variations in distribution of gross lipoid, of lipine and of substances coloured by Nile Blue and Nile Red in the adrenal cortex of albino rats. Evidence is produced of a rhythmical secretory change, irrespective of age, sex and spaying, and there is some evidence against the z. glomerulosa being considered as 'pre-secretory'.

Throughout this paper the word lipoid refers to all substances colourable by Sudan Black (see Lison, 1936) and the word lipine refers to lipoids containing fatty acid radicles and nitrogen or phosphorus or both, i.e. the phospholipines and galactolipines.

MATERIAL AND METHODS

Albino rats, all inbred from the same colony in the Department of Human Anatomy, were used for this investigation. Table 1 shows the different groups of rats, the number in each group and the body weights. The criterion of immaturity in the females was an unopen vagina; in males an upper limit of 30 g. body weight was taken.

Lipine was demonstrated by Baker's (1946) acid haematein method; his pyridine extraction control test gave consistently negative results for everything but nuclei and some blood corpuscles in individuals from each of the

above groups (Pl. 2, figs. 4, 5). For total lipoids Sudan Black and Sudan IV were used after fixation in Baker's (1944) formal-calcium, as for the acid haematein test but without post-chroming. Baker's (1944) modification of Lison's (1936) method for the lipoid colorants was used (see Cain (1947) for discussion).

Lorrain Smith's method for Nile Blue sulphate as given by Lison (1936) was used, but boiling with 0·5 % sulphuric acid was found to be unnecessary. Lison (1935*a*, *b*) has stated that neither the red coloration nor the blue staining from aqueous solutions of Nile Blue sulphate are specific for any class of lipoid; the red coloration is specific for lipoids in general, the blue for nothing at all, and the combination has no less, and no more, significance than any other combination of a lipoid colorant and a basic dye. These conclusions supersede those given in his book (1936). Work by one of us (Cain, 1947), while confirming Lison's results, shows that, provided a body is known to be lipoid, such a combination can be used to detect acidic lipoids in it. If it consists only of neutral lipoids (esters and hydrocarbons), it will be coloured red, if acidic lipoids (fatty acids, lecithin and probably other phospholipines) are present it will be coloured blue. These colorations are therefore offered in this paper as distinguishing two classes of lipoid under the conditions of the test. Baker's (1946) method for lipines is offered as a definite histochemical test. If, in future, this method is found to be not entirely specific, the results obtained with it still enable morphological distinctions to be drawn.

Schultz's method of application of the Liebermann-Burchardt method for cholesterol and its esters as quoted by Kay & Whitehead (1937) was also used.

RESULTS

Gross lipoids

(*a*) *Sudan Black and Sudan IV*

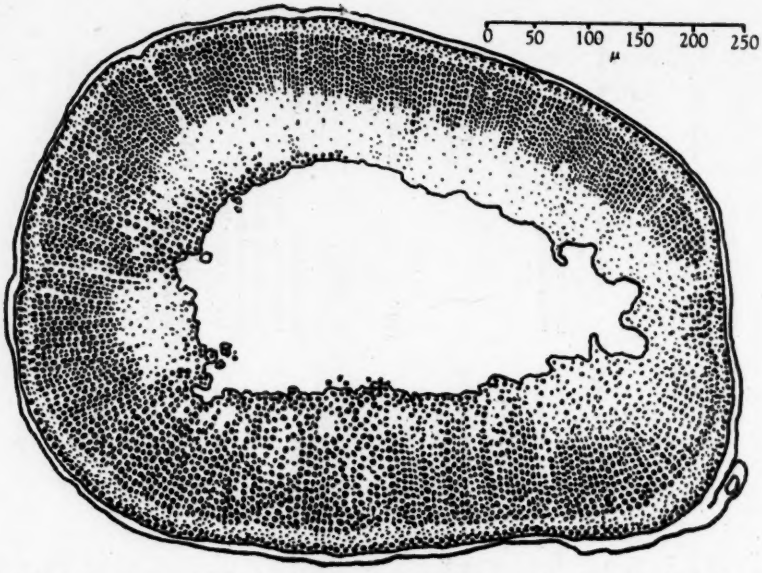
There is, as might be expected, very little difference between the results obtained from the Sudan Black and Sudan IV methods, except that with Sudan IV, because of the inevitable general pale coloration, which has no significance (Cain, 1947), the finer details in the less heavily loaded tissues are obscured. The more heavily loaded tissue is therefore more sharply delimited, the rest of the section being very evenly coloured (Pl. 1, figs. 1, 2). Consequently only Sudan Black preparations are described except where otherwise noted. With the Sudan Black method, either the outer part of the z. fasciculata was the most heavily loaded or there was even loading throughout the zone. In no case was the inner z. fasciculata more heavily coloured than the outer—a state of affairs to be described under the section on lipines. The z. reticularis, when present, was as dark as or less dark than the inner z. fasciculata, but never darker except for a small number of scattered and very heavily loaded cells next to the medulla in some individuals of most groups. The z. glomerulosa, on the other hand, was extremely variable; usually there was an inner zone

Table 1. *Summary of groups and some of the results*

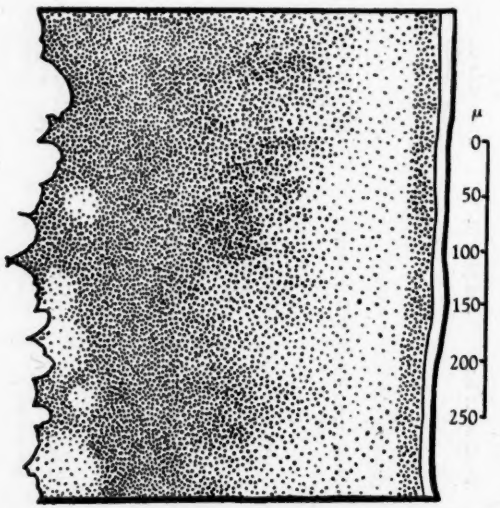
Animal group and no. in each group	Reticularis	Sudanophobe zone	Red in Nile Blue sections	Inmost zone with more lipoid than rest of reticularis	Glomerulosa most heavily loaded region	Outer fasciculata the most heavily loaded region of whole fasciculata	Abrupt edge to heavily loaded fasciculata
Immature males:							
Litter 1 (4 rats) (15–18 g.)	All small	Present	Very slight in reticularis	None	No	Yes, but even all through	None
Litter 2 (4 rats) (20–22 g.)	All small	Present	Very slight in glomerulosa	None	No	Yes, but even all through	None
4 other rats (24–27 g.)	Small, rather variable	Present	None in one, a little in one, very much in another	One only	One only	Yes, but even all through	None
Mature males (2 rats) (258–341 g.)	Large	Present	Present in large quantities	Scattered cells only	One slightly darker, one very much darker	Yes, but even all through	Rather faintly in one
Immature males 7 weeks after castration (2 rats) (172–196 g.)	Small to medium	Present in one, indistinct in others	Present	Distinct in one	Yes, in one, less distinctly in others	Yes, but even all through	Slight
Immature females:							
Litter X (4 rats) (22–30 g.)	Medium to small	Present	None	None	Some equal to fasciculata, others less	Yes	Occasional
Litter Y (3 rats) (20–22 g.)	Medium to small	Present	None	None	Some equal to fasciculata, others less	Yes	Occasional
Litter Z (3 rats) (27–30 g.)	Medium to small	Present	None	None	Some equal to fasciculata, others less	Yes	Occasional
Litter W (6 rats) (24–43 g.)	Medium to small	Present	None	Slightest trace in one individual	Some equal to fasciculata, others less	Yes, but not always the most peripheral region	Occasional
1 other rat (48 g.)	Medium	Present	None	Not obvious	No	Yes	No

Table 1 (*continued*)

Females in oestrus (4 rats) (149–217 g.)	Medium to large	Not present	Present in all, to varying extents	Scattered cells in 3, fairly definite ring in fourth	Definitely darkest in one. Less dark than fasciculata in one. Rest approx. = fasciculata	Yes, but reverse gradient in 2 acid haematein preparations	Yes, in two, very abrupt
Females in dioestrus (4 rats) (180–243 g.)	Medium to large	Not present	One with very little, rest with a great deal	Indistinct, not present in one	Much the darkest in three	Yes	Very distinct in two
Immature females 4 weeks after spaying (4 rats) (129–170 g.)	Medium to small	Present in 3, indistinct in others	In two only	Indistinct or definitely not present	No	Yes	Yes in one
Immature females 11 weeks after spaying (4 rats) (134–210 g.)	Medium to small	Present in 3 only	Some in two	Very conspicuous in one, indistinct in others	One only	Yes, but even throughout. Reverse gradient in one acid haematein preparation	Yes in one
Pregnant females (3 rats) (135–268 g.)	Small	Absent or indistinct	Small amounts in two	Indistinct	Yes	Even throughout. Reverse gradient in one acid haematein preparation	Rather faint in one

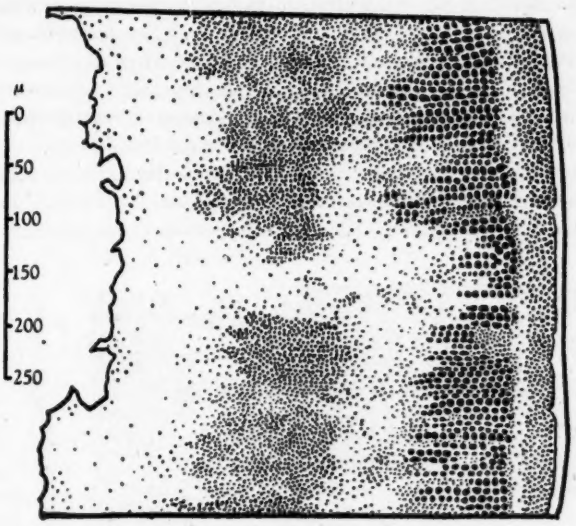


Text-fig. 1. Immature male. Section of whole adrenal. Acid haematein. Camera lucida outlines; medulla left blank. Stippling to show distribution and relative quantity of lipine. Note from the outside inwards, clear capsule, dark z. glomerulosa, clearer zone (corresponding to the sudanophobe zone in Sudan Black preparations), very heavily loaded outer z. fasciculata, inner z. fasciculata and reticularis relatively free except in patches.

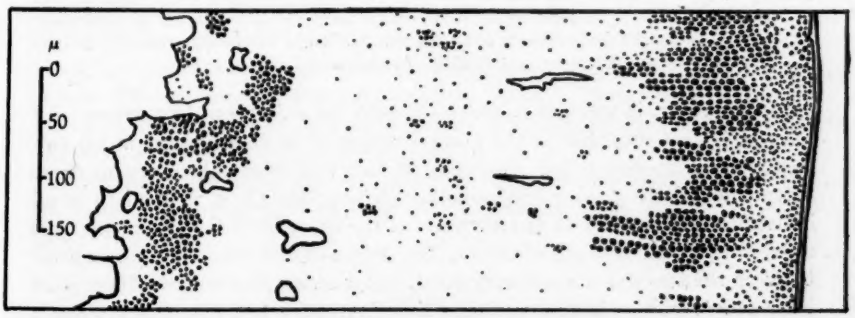


Text-fig. 2. Pregnant female. Portion of section through adrenal. Cortex only. Acid haematein. Z. glomerulosa moderately dark, sudanophobe zone merged into uncoloured outer z. fasciculata. Colouring increases inwards to the medulla. Z. reticularis rather patchy.

clear of lipoids which appears to be the same as the sudanophobe zone, referred to by many workers, and described by Deane & Greep (1946) as subglomerulosal (Pl. 1, figs. 3, 4). In these cases the outer zone was usually heavily loaded, in



Text-fig. 3. Mature female in dioestrus. Cortex only. Acid haematein. Note from the outside (right-hand side) inwards, clear capsule, medium dark z. glomerulosa, clear zone, very heavily loaded outermost z. fasciculata, fairly clear middle z. fasciculata, and darker inner. Z. reticularis almost unstained.

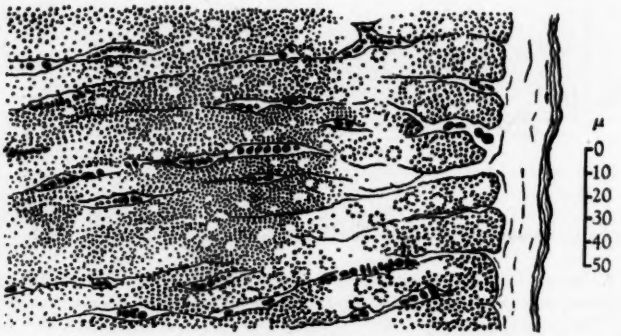


Text-fig. 4. Female, 11 weeks after spaying. Cortex only. Acid haematein. Z. glomerulosa moderately dark, sudanophobe zone not clearly defined. Outermost z. fasciculata heavily loaded, rest almost clear. Darkly coloured patches in z. reticularis.

several cases being often the darkest part of the whole section. Frequently, the z. glomerulosa was not present as a continuous sudanophil rim, but was often interrupted by sudanophobic patches; this discontinuity was also seen with the

lipines (Pl. 1, fig. 3 and Pl. 2, fig. 2). The sudanophobe zone could be seen in every immature male and normal female adrenal examined; it was also present, though not uniformly, in mature males and the two groups of spayed immature female rats. Apart from one section in the castrated male group, in which there was a slight separation of the outer, dark, z. glomerulosa from the z. fasciculata, the sudanophobe zone was seen in the adrenals of none of the rats of other groups (Pl. 1, fig. 5 and Pl. 2, fig. 1). It is, perhaps, significant that this sudanophobe zone could be found only in the groups indicated and that it was absent in the adrenals of normal adult or pregnant female rats.

The z. glomerulosa was darker than the z. fasciculata in some animals of all groups examined except immature normal females and the group of immature females 4 weeks after spaying. When this appearance was present in a given



Text-fig. 5. Enlargement of part of same section as Text-fig. 3. Acid haematein. Capsule, with darker outer layers, z. glomerulosa, sudanophobe zone, and outer part of z. fasciculata. Nuclei are completely unstained, cell walls not shown up, blood corpuscles very heavily stained. The fasciculate arrangement of cells extends to the capsule.

group, however, it was not uniform amongst the animals of that group. An interesting fact was that in the normal immature male group, in which two groups of litter-mates each consisting of four rats were taken, one of these litters showed the z. glomerulosa rather darker than the z. fasciculata in all individuals, whereas in all the members of the other litter the two zones were universally equal in depth of colour. The degree of difference in the first litter between the two zones is not very great, but it seems that whereas there is an inter-litter variation in this particular tissue's reaction to Sudan Black, there is an intra-litter uniformity: this is in close agreement with the findings with regard to organ weights as shown by Cameron, Guthrie & Carmichael (1946). It demonstrates the great care which must be taken in the interpretation of findings from a random selection of animals.

As has been mentioned, an innermost ring of scattered, heavily loaded cells could occasionally be seen in the z. reticularis bordering on the medulla in some

sections. It was seen in various animals of different groups but not in any immature normal females. Therefore this appearance can hardly be described as the *X*-zone (see Grollman, 1936); if it does correspond to the *X*-zone then clearly it cannot be universally present in immature rats and can, in some cases, persist even into pregnancy. The best demonstration of this appearance, which may be due to degenerating cells, was in an 11-weeks spayed animal.

In all groups the z. fasciculata either showed an even colouring or else the outer z. fasciculata was darker than the inner. In the latter case the outer, more darkly staining z. fasciculata existed as a rim with the rest of this zone showing even distribution of lipoid, or else there was a steady gradient from without inwards. In the case of the two litter-mate groups of immature males there was again seen to be intra-litter uniformity and inter-litter variation in the histological picture in this respect.

(*b*) *Nile Blue*

In general the depth of blue staining appears to follow that with Sudan Black but in several cases the z. reticularis, particularly its innermost part, appears to stain more deeply than might be expected, and in many cases there is a replacement, partial or, rarely, nearly complete, of blue staining by red in both z. fasciculata and glomerulosa. Replacement in the z. reticularis is usually slight, the greatest amount of red coloration being seen in the outer z. fasciculata or glomerulosa. In the case of some of the rats of the mature oestrus and dioestrus groups and in the immature female rats 11 weeks after spaying, there was a ring of massive red coloration in the outer z. fasciculata; this massive coloration was seen in none of the other groups. There was, however, a slight red coloration in the same region in most normal mature and castrated immature males and in some spayed females and one pregnant rat.

A red coloration could be seen in the z. glomerulosa in some of the rats of all groups except the normal immature female and pregnant female groups. Again, this appearance was only sporadic within a group. A patchy red coloration was present in the z. fasciculata and reticularis of some mature and castrated males and mature and spayed females.

There was some intra-litter similarity in the histological picture in the two litters of immature males, with an inter-litter difference between some members of the two litters.

Cholesterol

The cholesterol test was applied to individuals of all groups except immature and pregnant females. In all cases the z. glomerulosa and fasciculata contained cholesterol throughout, while the z. reticularis had none or at the most it was found in a very few scattered cells. In cases where the sudanophobe zone was seen in Sudan Black preparations, the corresponding region was found to be comparatively free from cholesterol.

Lipines

The distribution of lipine in general follows that of gross lipoids, as shown by Sudan Black, quite closely. The z. glomerulosa cells all show a certain amount of lipine, though where there is an inner sudanophobe zone, this is less coloured than the outer part of the z. glomerulosa (see Text-fig. 1 and Pl. 2, fig. 2). (It should be remembered when comparing Sudan Black and acid haematein preparations that acid haematein is the more powerful stain, often colouring mitochondria in other tissues blue-black when Sudan Black only tinges them grey.) Where the z. glomerulosa is the darkest in Sudan Black sections, in acid haematein sections it usually appears less dark in comparison, and in some cases may be a little paler than the darkest patches in the z. fasciculata.

Usually the outer region of the heavily loaded z. fasciculata is well coloured in acid haematein sections (Pl. 2, fig. 3), but in a few cases, among mature and spayed females the lipine is scanty in the outer part of the z. fasciculata, and actually increases a little inwards towards the medulla (see Text-fig. 2 and Pl. 2, fig. 6)—a gradient not seen in any other preparation, though in some Nile Blue sections the z. reticularis is of a rather darker blue than the inner part of the z. fasciculata. This gradient is seen throughout the section only in a pregnant individual; in the rest the z. fasciculata is very patchy. In one individual, a female in dioestrus (Text-fig. 3), there is a lighter band running through the middle of the z. fasciculata.

The z. reticularis sometimes has more lipine than might have been expected in the region next the medulla. The part next the z. fasciculata is usually very poor in lipine—the poorest region in the section—except in those cases mentioned above where a reverse gradient appears. But in most sections there are scattered cells next the medulla which are quite well coloured and in a few females a definite well-coloured inmost zone is seen in acid-haematein sections; and indications of it are seen in other sections in one immature male, and one castrate. It was seen best in an 11-weeks spayed individual (see Text-fig. 4).

To sum up, very few cells appear to be completely without lipine; those that are so deficient occur in the z. reticularis. In general, the distribution of lipine is proportional to that of total lipoid except in the sudanophobe zone of the z. glomerulosa, where present, and in a few cases in the inner regions of the cortex where gross lipoid is absent. However, it is noticeable when acid haematein and Sudan Black sections of adrenals of the same individual are compared that although the outer border of the heavily loaded outer z. fasciculata occurs in much the same place in both, the inner border is sometimes much nearer the medulla in Sudan Black preparations than in acid haematein ones. If, as several writers believe (see Smedley-Maclean (1948) for review and discussion), lipine is concerned in the secretion of fats, it would seem that in the z. fasciculata it is the outermost region only which should be called the secretory zone, as far as fats are concerned, and in the contiguous region towards the medulla secretion is reduced or stopped, but dispersal of the products

has not yet taken place. Bennett (1940) describes the whole of the lipoid-rich zone of the z. fasciculata as containing ketosteroids, and therefore calls it 'the secretory zone'. It appears from the work described in this paper that possibly only the outer part of this zone is secretory for fats, and the inner part may be considered post-secretory for fats but not for ketosteroids.

DISCUSSION

A. *General structure*

There are usually only three of Bennett's four zones to be seen in the rat adrenal, the post-secretory zone being seldom clearly marked, as Bennett has noted for various other animals. Our results do not contradict his observations in general except that, in adult males, the secretory zone (distinguished by its high lipoid content) usually occupied more than half of the cortex. Although the inner z. fasciculata and z. reticularis are usually smaller in proportion in immature and spayed animals, there is considerable variation. An examination of many more individuals is necessary before any definite conclusions can be drawn.

Detailed examinations of the zones will be discussed in a future paper, but one point must be mentioned here. Bennett (1940) states that the pre-secretory zone may on occasion be absent altogether, in which case the z. fasciculata comes right up to the capsule; and in his description of this zone he mentions that its cells are comparatively poor in lipoid, and that they grow richer as they pass inwards. In the rat, the outermost region, as defined by Sudan Black preparations, is exceedingly variable in this respect, but rarely, if ever, gets richer in lipoid towards the inside. It is of fairly constant depth (5–8 cells usually) and in males, castrates, and some spayed females the inner half is definitely sudanophobe, but the outer is not and may be as dark in Sudan Black preparations as the adjacent part of the secretory zone. In adult females and some others, there is no such clear distinction, and colouring appears even throughout. In some cases, in both sexes, the zone, whether divided or not, is the darkest part of the whole section in Sudan Black preparations. Some cases were observed in which the z. glomerulosa appeared to have vanished and the z. fasciculata had come up to the capsule (see Text-fig. 5); but in these cases the outermost region, again to the depth of 5–8 cells, is still differentiated by its sudanophil content. Even if the fasciculation does extend right up to the capsule and the subcapsular cells are sudanophil, it does not necessarily follow that such cells correspond to the z. glomerulosa, although in some cases, as will be seen from Text-fig. 5, this region, when there is complete fasciculation, appears to include the capping cells of each column, which are the z. glomerulosa in the strict sense, and cells in the z. fasciculata. Bennett makes it clear that his presecretory zone not only contains no ketosteroids but also is poor in lipoids and adds that in some animals it is entirely lacking. It seems, therefore, that no corresponding zone has been seen in these experiments.

B. *Variations in staining and coloration*

It will be seen from Table 1 that red coloration with Nile Blue is found in some individuals in every group except immature females. It is very extensive in the mature males and most of the females in dioestrus, and very scanty in immature males. The abrupt change from heavy to light loading in the z. fasciculata is seen in some individuals of several groups; and in some a very heavily loaded z. glomerulosa is seen.

These characters do not appear to be correlated with the grouping of the animals. Bennett considers that variations in zoning in adult females may be correlated with the oestrous cycle and quotes Andersen & Kennedy (1932) in support. Our evidence suggests that in the rat at least there may be variations in immature animals as well. Deane & Greep (1946) noted that in hypophysectomized animals the z. glomerulosa may be the most heavily loaded zone. Among our rats it appears to be so in several groups. The variation in red-staining and blue-staining material has, as far as we know, not been reported before.

A comparison of the two litters of immature males gives very interesting results. In one litter, only two individuals show any red at all, and both do so only in a few cells of the z. reticularis next the medulla. In the other, again two show a very little red, but it is in the z. glomerulosa. Of the miscellaneous immature males, one has preponderance of red in the z. glomerulosa, and a slight quantity in the rest of the cortex. Another, agreeing with the first litter in having no red, disagrees with both in showing an inmost zone of heavily loaded cells. With so much variation in four immature males taken at random, the comparative lack of variation between litter-mates and the difference between the two litters are very interesting. It is possible that there is some sort of rhythmic change in the adrenals here as well as in mature females. Since there is considerable intra-litter similarity, it is possible that individuals of a given litter are all at much the same stage of a given cycle. Accordingly, four litters of immature females were used for further investigation. The animals of each of three litters (X, Y and Z in Table 1) were killed simultaneously: the animals of the fourth litter (W) were killed at the same time on successive days. By this means it was hoped to show greater variation between than within litters with X, Y and Z. The animals of the W litter, it was hoped, would show successive stages of the cycle, since they were killed over a period of 6 days. The adrenals of X, Y and Z litters showed great similarity, in general, to those of immature males, an equally great intra-litter similarity and even greater inter-litter similarity. However, the acid haematein preparations of the W animals provide a possible explanation for this. The first showed a narrow, darkly stained zone in the outermost z. fasciculata, the rest of this zone and the z. reticularis being very clear. In the second this dark zone, though still narrow, had its heaviest loading towards the medulla and in some places the part of the z. fasciculata next to the sudanophobe zone was itself clear. In the

next two the darkly stained zone was definitely wider, occupying more than half the width of the cortex and was very evenly coloured throughout. The fifth, in contrast to all the preceding, showed a very general coloration throughout the z. fasciculata and reticularis, the outermost z. fasciculata being rather the darkest; individual cells varied greatly, some scattered singly throughout the cortex being almost black. The sixth resembled the first extremely closely, but there was a slight trace of darkening in the inmost cells of the z. reticularis, not a trace of which was seen in any of the others. It would appear, therefore, that the main discharge of lipine, at least, takes place comparatively rapidly, and that the onset of the following cycle takes place equally rapidly. In all the adrenals of the immature females examined there was a sudanophobe zone which, in some cases, was almost double its normal width, notably in the second rat of litter W. In no case was the z. glomerulosa darker than the rest of the cortex, although often as dark as the outer z. fasciculata. Much the same picture is given by the other preparations, but it is obscured by the greater mass of total lipoid present.

SUMMARY

1. In the adrenal cortex of the rat there is considerable variation in the quantity and distribution of lipine, of gross lipoid, and of substances colouring red with Nile Blue. No corresponding variation in cholesterol is seen; this substance appears to be present always in the z. glomerulosa and z. fasciculata, and absent or nearly so from the z. reticularis. After staining for lipoids, three main regions are distinguishable in the cortex:

(*a*) The outer region contains the z. glomerulosa plus a small part of the z. fasciculata. (The latter may or may not be sudanophobe in part.) This region is very variable indeed with respect to lipoids.

(*b*) The middle region is the heavily loaded part of the z. fasciculata. It corresponds to Bennett's secretory zone, but it is possible that lipoids other than steroids are secreted in the outer part only, and merely stored prior to dispersal in the inner.

(*c*) The inner region is the rest of the z. fasciculata plus the z. reticularis, and is usually poor in lipoid, except in some of the cells of the z. reticularis. This corresponds to the post-secretory plus senescent zones of Bennett.

2. Some of the variations observed are not correlated with age and sex. In two litters of immature males examined, intra-litter variation was slight, inter-litter variation more marked. There was greater similarity throughout in three litters of immature females. These three litters taken in combination with a fourth, the members of which were killed on successive days, show some evidence for a cyclic change consisting of a long period of secretion and a much shorter period of discharge, if the hypothesis that individual members of a litter are at the same stage of the cycle is justified; the period appears to be of the order of five days.

3. Bennett's term 'pre-secretory zone' for the region corresponding roughly to the z. glomerulosa plus a little of the z. fasciculata is not appropriate in

certain cases in which it is the most heavily loaded region in the section. When little lipoid is present in it, the less loaded region is always the inner half; the outer, next the capsule, is always comparatively well loaded, and usually nearly as much so as the outer z. fasciculata, sometimes more so. There is seldom or never an increase in lipoid from the capsule inwards. This seems to agree with Deane and Greep's suggestion that this region is, at least partly, an independent secretory zone.

We wish to thank Dr J. R. Baker for valuable criticisms and Mr L. G. Cooper for technical assistance.

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EXPLANATION OF PLATES

Plate 1

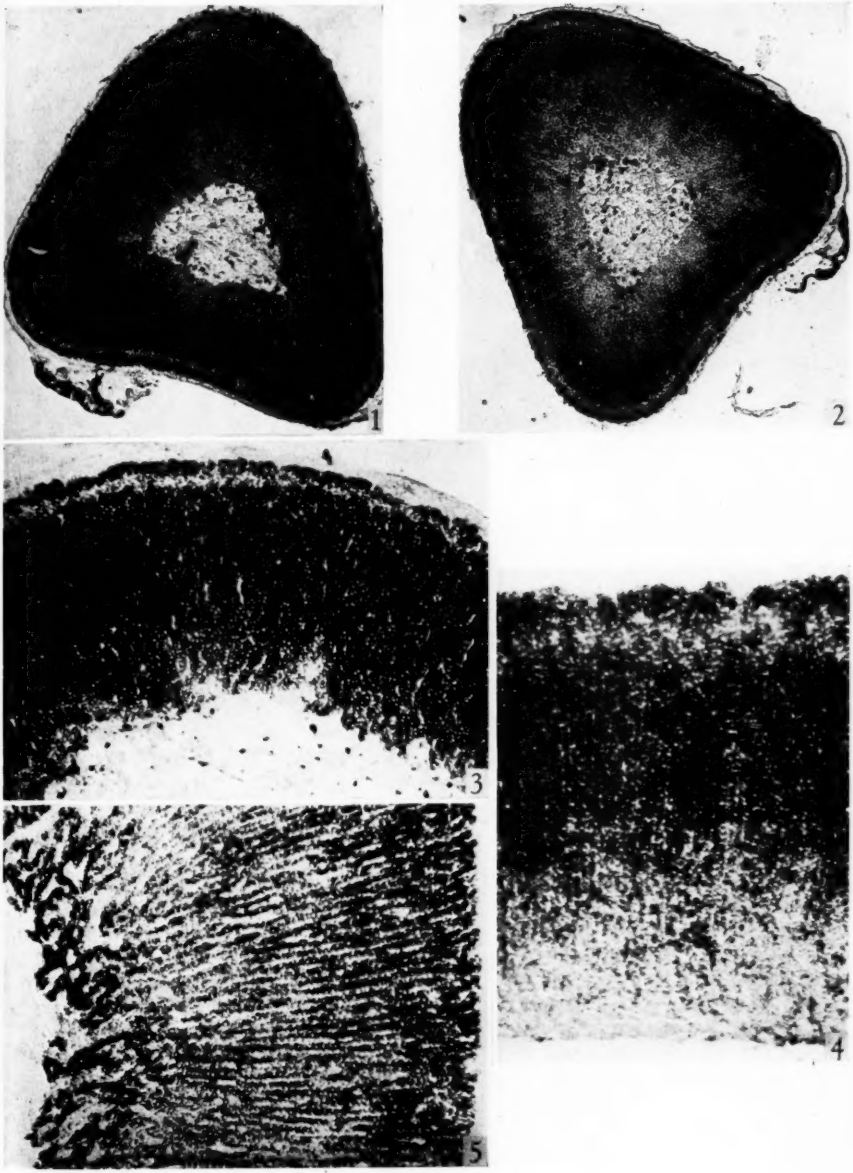
Fig. 1. Complete section of adrenal of an immature male rat. Sudan Black. (×55.)

Fig. 2. Complete section of the same adrenal as in fig. 1. Sudan IV. (×55.)

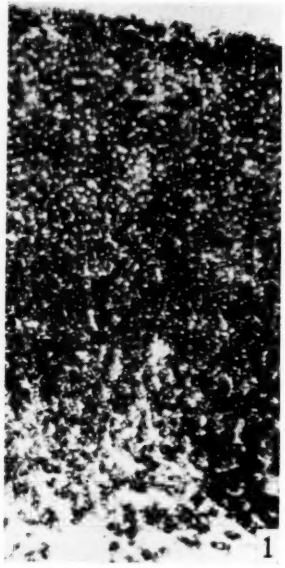
Fig. 3. Adrenal cortex of immature male rat. Sudan Black. Well-defined sudanophobe zone. Fasciculation not very obvious and not extending into z. glomerulosa. Little or no z. reticularis. (×98.)

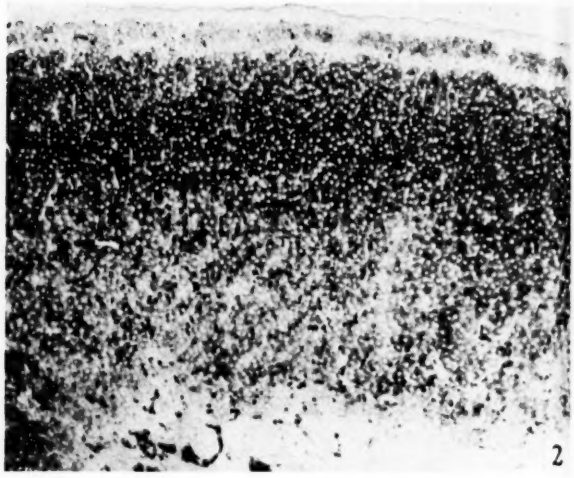
Fig. 4. Section of adrenal cortex of mature male. Sudan Black. There is good fasciculation which does not extend markedly into the z. glomerulosa. A sudanophobe zone is present. Large z. reticularis. (×98.)

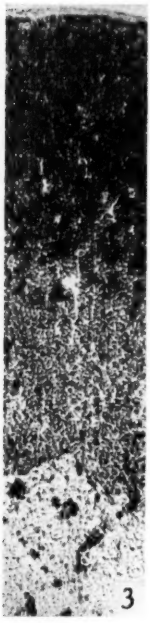
Fig. 5. Adrenal cortex of mature dioestrous rat. Sudan Black. Showing extreme fasciculation which extends into the z. glomerulosa. No sudanophobe zone. Well-defined z. reticularis. (×98.)

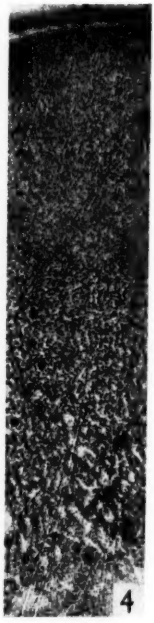


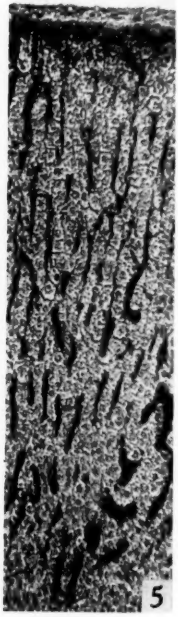
HARRISON AND CAIN—THE ADRENAL CORTEX OF THE ALBINO RAT











HARRISON AND CAIN—THE ADRENAL CORTEX OF THE ALBINO RAT

PLATE 2

Fig. 1. Adrenal cortex of pregnant rat. Sudan Black. Absence of sudanophobe zone. Z. glomerulosa much the darkest part. Large z. fasciculata and reticularis. (× 98.)

Fig. 2. Adrenal cortex of immature male rat. Acid haematein method. Showing same appearance as in Pl. 1, fig. 3, except for more marked z. reticularis. Note the small dark-coloured bodies (blood corpuscles) between the columns of the z. fasciculata. (× 98.)

Fig. 3. The adrenal cortex of an immature female four weeks after spaying. Acid haematein method. The colouring is mainly restricted to the outer z. fasciculata and glomerulosa and is not patchy. (× 55.)

Fig. 4. A control mature male adrenal cortex treated by pyridine extraction before acid haematein staining. Note, in contrast to fig. 6, the general absence of colouring. (× 55.)

Fig. 5. As Pl. 2, fig. 4, but at a higher magnification. Note again the dark staining in the line of the blood vessels in the cortex. (× 135.)

Fig. 6. Section of adrenal of a rat in dioestrus. Acid haematein method. Note how the inner z. fasciculata is coloured more darkly than the outer. (× 55.)

OBSERVATIONS ON THE ANATOMY OF THE FOSSIL AUSTRALOPITHECINAE

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The Australopithecinae, a sub-family of the Hominoidea originally created by Gregory & Hellman (1939), include all those fossil ape-like creatures whose remains have been recovered from limestone deposits in South Africa and which are at present allocated to the three genera *Australopithecus* (Dart), *Plesianthropus* (Broom) and *Paranthropus* (Broom). The present writer has recently had the opportunity and privilege of seeing this material during a short visit to Johannesburg and Pretoria and, owing to the generous facilities provided by those who were concerned with its actual discovery, Prof. Raymond Dart and Dr Robert Broom, he has been able to study it in some detail. In the present paper, some of the results of this examination are presented. In view of the numerous publications which have already appeared in reference to the Australopithecinae, and particularly the comprehensive monographic study recently published by Broom & Schepers (1946), it might be deemed superfluous to submit a further report. However, this appears justifiable for two reasons. In the first place, it has now become apparent that the fossils are of quite paramount importance in relation to problems of human phylogeny, and it is, therefore, hardly possible to overemphasize their significance. Secondly, it has to be admitted that, in the past, several anatomists of recognized distinction have, by their misinterpretation of the evidence, tended to belittle their importance. Even in some recent publications dealing with human palaeontology, e.g. the latest edition of Boule's '*Les Hommes Fossiles*' edited by Vallois (1946), their significance is evidently understated. The observations recorded in this paper are partly supplementary to those of Dart and Broom, and partly a re-emphasis of details which have already been mentioned by those authorities and which seem to the present writer of such special significance as to merit renewed attention.

As is now well known, the first representative of the Australopithecinae to be discovered was *Australopithecus*. This genus is represented by the skull and natural endocranial cast of a young individual which were discovered at Taungs in Bechuanaland and described by Dart in 1925. In 1936 and during subsequent years, remains of adult Australopithecines were found by Broom at Sterkfontein and Kromdraai, two places close to Krugersdorp and only two miles from each other. These remains, which include portions of skulls, natural endocranial casts, jaws, teeth and fragments of limb bones have been allocated by Broom to two separate genera, *Plesianthropus* (from Sterkfontein) and *Paranthropus* (from Kromdraai).

For convenience of reference, the following list is given of the parts of Australopithecine skulls found by Dr Broom. The type specimen of *Plesianthropus* consists of the whole of the base of the skull (with a natural endocranial cast in position), a considerable part of the facial skeleton and palate, a portion of the cranial roof (including a negative cast of the latter in the limestone matrix), and a fragment of the squamous portion of the occipital bone. Other material from Sterkfontein includes an excellently preserved right maxilla with some of the teeth *in situ*, a much crushed left maxilla with a portion of the zygomatic bone attached, the symphysial part of an immature mandible, and the angular region of the mandible of an adult individual. The type skull of *Paranthropus* consists of the left maxilla and zygomatic bone, a considerable part of the palate, the temporal and sphenoidal regions of the skull wall, the zygomatic arch, the glenoid fossa, the external auditory meatus, and part of the occipital bone. The type mandible includes the body of the right side with the molar and premolar teeth, the lower end of the vertical ramus, and a (separated) symphysial fragment. In addition, the mandible of a young individual with the milk dentition excellently preserved was found embedded in the limestone matrix not more than 4 ft. from the type skull.

A systematic account of all the fossil Australopithecine remains has been given by Broom & Schepers in their recent monograph. From this account, the following main points emerge. The Australopithecinae had quite small brains, approximating in size to those of the gorilla and chimpanzee, and massive jaws with large molar and premolar teeth but relatively small incisors and canines. The dental arch is evenly curved in parabolic form (thus contrasting very strongly with the recent anthropoid apes), and the molar teeth show a type of wear due to attrition apparently identical with that characteristic of man. In the construction of the supra-orbital region, the details of temporo-mandibular articulation, and the disposition of the foramen magnum (as well as several other cranial characters), the Australopithecine skull shows a definite approach to the human skull. Lastly, the limb-bone fragments, particularly the lower end of the humerus of *Paranthropus*, and the lower end of the femur of *Plesianthropus*, seem to indicate a limb structure which evidently approximated very closely indeed to that of *Homo sapiens*. This evidence of the limb bones is sufficiently startling in character to raise a doubt as to whether they actually belonged to the same creatures as the skulls of *Plesianthropus* and *Paranthropus*. That the association is entirely correct, however, seems quite well assured as will be seen in a later section (p. 318). On the basis of the evidence presented by the first announcement of the discoveries of the Australopithecine fossils, there seemed at first sight to be several possible interpretations. The Australopithecinae might be nothing more than extinct varieties of ape closely akin to the chimpanzee and gorilla, but with certain modifications which in some minor respects show a spurious resemblance to the Hominidae. Secondly, they might

* Since this was written Dr Broom has announced further important discoveries of *Plesianthropus* material.

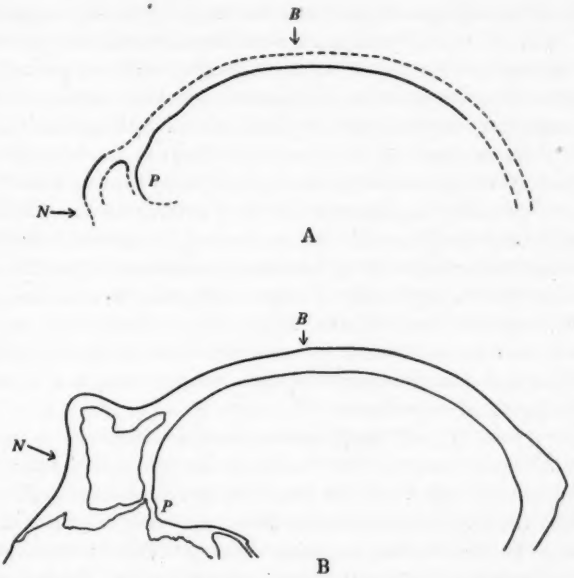
have no special relationship to the gorilla and chimpanzee, but, nevertheless, represent a collateral group of anthropoid apes showing certain human characters developed as the result of a parallel evolution but not necessarily indicative of any real affinity with the Hominidae. Lastly, the Australopithecinae could be regarded as extinct hominoids which, while still at (or, at least, close to) the simian level in their cerebral development, were early representatives of the human branch of evolution and thus quite distinct from the Pongidae. This last interpretation has for a number of years been reiterated by Dart and Broom. On the other hand, other anatomists and palaeontologists (particularly those who have not had the advantage of examining the original fossil material) have, either by direct statement or by implication, favoured one of the first two interpretations. As the result of his personal studies, the present writer has come to the firm conclusions (1) that the Australopithecinae have no special relationship to the recent anthropoid apes except so far as they are large hominoids of comparable size, and (2) that the human resemblances in the skull, dentition and limb bones are so numerous, detailed and intimate as virtually to preclude the introduction of the idea of 'parallel evolution' in order to explain them. In other words, there must be a real zoological relationship between the Australopithecinae and the Hominidae.

For the main descriptive details of the Australopithecine material, the reader is referred to the many publications of Dart and Broom (see the bibliography ni the monograph by Broom & Schepers, 1946). As already mentioned, the present communication is concerned not with a systematic account of these fossils (which would be unnecessary), but with certain points which appear to be of special significance.

THE SKULL OF THE AUSTRALOPITHECINAE

Frontal and supra-orbital regions. Dart has already called emphatic attention to the human characters of these regions in the skull of *Australopithecus*. The details of the supra-orbital region in this fossil are particularly impressive, for they are quite unlike those of the recent large anthropoid apes (at any stage of growth). On the contrary, they almost precisely reproduce those of *Homo sapiens* in the evenly rounded contour of the supra-orbital eminence which lies above the medial half of the supra-orbital margin (here blunt and rounded) and its replacement laterally by a shallow depressed area bounded below by a sharp orbital margin. The appearance of the zygomatic process of the frontal bone likewise recalls that of the human skull. The relationship of the frontal pole of the brain to the nasion and supra-orbital region in the young *Australopithecus* skull has been discussed by Sollas (1926), who drew attention to certain remarkably human features which it presents. In the adult *Plesianthropus* the relationship can be determined quite clearly, for in the type skull the endocranial cast (which is practically perfect in the frontal lobes) fits accurately against the base of the skull and the latter includes the supra-orbital region

(see Pl. 1, fig. 3). In this region the summits of the natural casts of the frontal sinuses are preserved *in situ* and the position of the glabella can be identified. From these data it was possible with considerable accuracy to reconstruct the sagittal section shown in Text-fig. 1A.* If this reconstruction is compared with the sagittal section of a gorilla's skull shown in Text-fig. 1B, the contrast in the supra-orbital contours becomes at once apparent, and also the contrast in the relationship of the frontal pole of the cerebral hemisphere (*P*) to the nasion (*N*).



Text-fig. 1. A. Sagittal section of the calvarium of *Plesianthropus*, based on the relation of the endocranial cast to the base of the skull in the type specimen. Note the relation of the frontal pole (*P*) to the nasion (*N*) and the frontal air sinus. The position of the bregma is indicated (*B*). The continuous lines represent the contour of the endocranial cast and the preserved portion of the frontal air sinus and supra-orbital ridge. B. Sagittal section of the calvarium of a female gorilla for comparison with that of *Plesianthropus*. Both sections are shown at half natural size.

Similar contrasts, though less in degree, are also to be seen in comparisons with adult male chimpanzee skulls. In *Plesianthropus* the frontal pole of the cerebral hemisphere reaches forwards to a level not more than 17 mm. from the maximum prominence of the glabella, and the upper limit of the frontal air sinus is about 15 mm. above the level of the nasion, while laterally the supra-orbital ridges form an independent arch on each side and do not unite into a single, straight,

* This sagittal section is almost certainly more accurate than that obtained from the negative cast of the cranial vault in the limestone matrix, for while this cast may have been somewhat distorted by post-mortem pressure, the frontal region of the endocranial cast is quite undistorted.

uninterrupted horizontal torus. Indeed, the whole frontal region of the skull in section shows a remarkable resemblance in its contour to that of primitive representatives of the Hominidae. The frontal and supra-orbital regions of the skull of *Paranthropus* are not known but, as will be noted later, the appearance of the frontal process of the zygomatic bone in this fossil makes it very improbable that an exaggerated type of supra-orbital torus was present.

The lack of a strongly developed supra-orbital torus in the adult Australopithecinae is not only attested by the evidence of the mature skulls of *Plesianthropus* and *Paranthropus*; it may also be inferred from a consideration of the immature skull of *Australopithecus*. There is no sign in this specimen of an incipient supra-orbital torus; yet, at the same stage of development (i.e. at the time of the eruption of the first permanent molar) the supra-orbital torus is already becoming evident in those anthropoid apes (chimpanzee and gorilla) which have a strong torus in the mature skull. It is also interesting to note (Le Gros Clark, 1940) that an incipient torus is already evident in the Modjokerto skull (almost certainly an immature skull of *Pithecanthropus* of about three years of age), and that even in the immature Neanderthal skull found by Prof. Dorothy Garrod in 1926 at Gibraltar, estimated to be that of a child about 5 years old, the supra-orbital ridges characteristic of Neanderthal man had already begun to form (Keith, 1931). It seems desirable to emphasize these points, since in some tentative reconstructions of the complete skulls of *Plesianthropus* and *Paranthropus* the supra-orbital torus has evidently been given an exaggerated importance.

Zygomatic region. In all the Australopithecine skulls the zygomatic bone shows certain very noteworthy features. In the first place, its general orientation contrasts rather strongly with that found in all recent large anthropoid apes. In the latter, the facial surface of the bone looks not only laterally but also forwards to a considerable degree, being shunted in this direction as the result of the lateral prominence of the zygomatic arch behind it. Further, this surface slopes forwards gradually into the plane of the facial surface of the maxilla, without any abrupt change of inclination. In *Australopithecus* the facial surface of the zygomatic bone is orientated as in the human skull—its main part being directed laterally, and is thus angulated rather abruptly at the zygomatico-maxillary suture with the infra-orbital part of the maxilla which is here directed mainly forwards. If the fossil skull is compared with recent anthropoid ape skulls of corresponding dental age, the contrast is impressive. But the same feature is also to be observed in the adult skulls of both the other Australopithecine genera. In *Australopithecus* the shape and contour of the zygomatic bone is astonishingly human, and there can be very little doubt that, if the zygomatic portion alone of the skull had been found, it would certainly have been referred to *Homo*. Particular reference may be made here to the slenderness of the frontal process, the contour of its posterior border, and the appearance of the zygomatic tubercle. There is another significant point (to which Dart has already called attention). In the African apes—particularly young individuals

—a pronounced ridge (the torus zygomaticus) commonly extends downwards and a little backwards from the lateral extremity of the supra-orbital torus across the temporal surface of the zygomatic bone to the lateral end of the inferior orbital fissure. This simian feature is entirely absent in *Australopithecus*.

In *Australopithecus*, the slender frontal process of the zygomatic bone leads up into a correspondingly slender zygomatic process of the frontal bone, and (as might perhaps have been inferred without further evidence) there is no supra-orbital torus of the type found even in young individuals of the African apes. A careful examination of the facial part of the adult skull of *Paranthropus* shows that here too the frontal process of the zygomatic bone is flattened and smooth, lacking the roughened and robust character of the process usually found in the chimpanzee and gorilla where it is continued up into a strong supra-orbital torus. It thus seems certain (as already indicated) that a torus of this character could hardly have been present in *Paranthropus* (and we know from direct evidence that it was certainly absent in *Plesianthropus*).

The orbital border of the zygomatic bone in *Australopithecus* presents yet another curiously human feature in the degree of its backward curvature, which leads to a relatively marked recession of the lateral margin of the orbit. This recession is undoubtedly related to the recession of the facial region as a whole, and this same factor also determines the relative position of the temporal process of the zygomatic bone. In the recent anthropoid apes, the whole bone has the appearance of being drawn downwards and forwards (relative to the calvarium) by the downward and forward growth of the massive jaws, and the level of the temporal process is usually well below the level of the inferior orbital margin. In *Australopithecus* (as in man), the upper border of the temporal process at its junction with the posterior border of the main part of the zygomatic bone is approximately at the same horizontal level as the infra-orbital margin, and this appears also to have been the case both in *Paranthropus* and *Plesianthropus*.

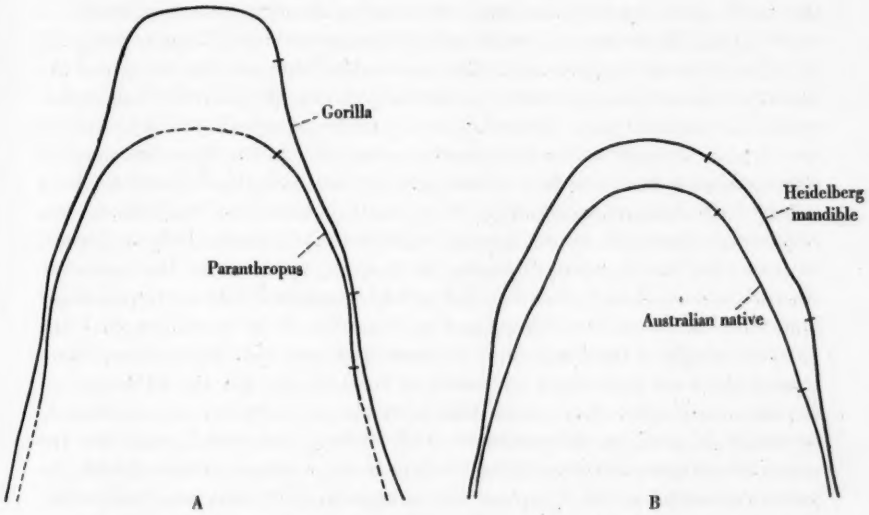
Maxillary region. Broom has noted a number of human characters in the anatomy of the Australopithecine maxilla. The slope of the infra-orbital region of the facial surface which looks predominantly forwards may be compared with the typical simian condition in which this surface looks more antero-laterally. This contrast is clearly related to the difference in the relative development of the canine and incisor region of the upper jaw. The infra-orbital foramen in all three genera appears to be single as in man (and not multiple as in recent apes). Moreover, it is somewhat smaller than the main foramen in adult gorillas and chimpanzees (its maximum diameter is 5·0 mm. in the *Paranthropus* maxilla, and 5·0 and 3·5 mm. in each of two *Plesianthropus* maxillae). The question of the existence of a facial portion of the maxillo-premaxillary suture is of some interest in relation to the emphasis (probably somewhat exaggerated) which some comparative anatomists have placed on this feature in the assessment of man's relationship to the lower primates. There is no sign of this suture in the

Paranthropus skull, and (unlike Broom) the present writer was unable to detect it with certainty in the *Plesianthropus* maxillae (though there is a distinct trace of the palatal portion of the suture in the female skull). In *Australopithecus* Broom believes there is a trace of the facial part of the suture in its upper part at the side of the nasal aperture. But the trace is exceedingly faint, and is probably not distinct enough for certain identification. Certainly, as Broom says, there is no sign of the lower part of this suture on the face, but this part becomes obliterated at a very early age even in the chimpanzee and gorilla. The palatal portion of the premaxillary suture is quite distinct in the *Australopithecus* skull.

The region of the temporo-mandibular joint. The nature of the wear of the teeth provides clear enough evidence that the mechanism of the temporo-mandibular articulation in the Australopithecinae must have been very different from that which is characteristic of the recent anthropoid apes. This inference is borne out in a striking manner by an examination of the *Paranthropus* skull. Dr Broom has already emphasized the human characters of the glenoid cavity in this fossil specimen (and his observations can be fully confirmed by anyone who examines the original fossil), but because of its important implications, a further brief reference may be made to it here. The 'hollowed-out' character of the glenoid fossa, bounded anteriorly by a transversely disposed, rounded eminentia articularis, reproduces the human condition very closely and is very unlike the broad flattened glenoid surface in the gorilla or chimpanzee. In both these apes also (but particularly in the gorilla), the articular surface is prolonged downwards at its medial margin into a strong and rather prominent lip, but this, as in human skulls, is only slightly indicated in *Paranthropus*. The post-glenoid process, which is so powerfully developed in modern apes, is represented in *Paranthropus* by quite a small tubercle of remarkably human appearance. The tympanic bone is thus well exposed below the process and presents a flattened anterior surface which actually forms throughout the whole of its extent (as in man) the posterior wall of the glenoid fossa.

Mandible. The only adult Australopithecine mandible sufficiently complete for study is that of *Paranthropus*. Broom has emphasized the massiveness of this bone and has also indicated some of its human features. The contour of the body of the mandible is certainly quite unlike that of any of the recent anthropoid apes, a fact which is sufficiently emphasized by the diagrams in Text-fig. 2. These diagrams show the horizontal curve of the outer surface of the body and angular region of the mandible in *Paranthropus*, a male gorilla, the Heidelberg mandible (taken from a cast) and the jaw of a megadont Australian. The curve was recorded by moulding a lead strip against the outer surface of the mandible at a level approximately midway between the alveolar and lower borders. In the case of the *Paranthropus* jaw, it was necessary to reconstruct the symphysial and angular regions. The symphysial region can be restored with sufficient accuracy by reference to the alinement of the teeth (particularly in relation to the palatal arcade which is accurately known) and to a small fragment of the

symphysis containing the sockets of the incisors as well as the root of the canine tooth (see Broom's Fig. 100, Pl. X, in his recent monograph). The angular region may have extended back a few millimetres farther than the diagram indicates. The extent of the premolar-molar series is indicated in all the diagrams. It will be apparent that, although the length of the premolar-molar series of teeth in *Paranthropus* is closely comparable with that of the male gorilla, the contours in the two specimens are very different. On the other hand, the contour of the *Paranthropus* jaw coincides to a remarkable degree with that of the Heidelberg jaw, even though in the latter the length of the premolar-



Text-fig. 2. Contours of the lower jaw (approximately mid-way between the alveolar border and the lower border of the mandible) of a gorilla, *Paranthropus*, the Heidelberg jaw, and an Australian native. $\times \frac{1}{2}$. In each contour, the levels of the anterior surface of the first premolar tooth and the posterior surface of the third molar tooth are indicated. Note the remarkable correspondence between the contour of the *Paranthropus* and Heidelberg jaws, though the extent of the premolar-molar series is very different.

molar series is very much abbreviated. It is to be noted that in the *Paranthropus* jaw the large molar teeth are accommodated by the extension of the aveolar border backwards for some distance under cover of the ascending ramus of the mandible. Thus, in lateral view, the last lower molar and a portion of the second molar are not visible. There can be little doubt, also, that Broom's reconstructions of the Australopithecine skulls are correct in indicating a rather high ascending ramus of the mandible. This again is secondary to the relatively forward position of the temporo-mandibular joint. It is interesting to note that in their second reconstruction of the *Plesianthropus* skull, Gregory & Hellman (1945) have evidently aimed at making the contour of the mandible

conform more closely to the usual simian type, but they have only been able to do this by pushing back the temporo-mandibular joint beyond its real position in relation to the base of the skull. One other point regarding the mandible which may be noted here is the trace of a distinct lingula overlapping the inferior dental foramen in the young *Australopithecus* skull. This process is absent in a number of young chimpanzee skulls which have been examined, and also (except to a rudimentary degree) in young gorilla skulls.

The occipital region. The position and orientation of the foramen magnum are of considerable importance in assessing the evolutionary status of the Australopithecine fossils. Fortunately, there are indications of its position in the base of the skull in all three genera, and these indications are quite consistent in showing that the foramen is placed definitely more anteriorly than in the skulls of the modern anthropoid apes. The ex-occipital bone and the margin of the jugular foramen are identifiable in *Australopithecus*, the posterior half of one occipital condyle is quite distinct in the *Paranthropus* skull, and the condyles are present (though rather indistinctly preserved) in the type specimen of *Plesianthropus*. Dart and Broom have already affirmed the forward displacement of the foramen magnum in their fossil material and emphasized this remarkable approach to the human condition. It is particularly important to note that the forward displacement is quite apparent in the immature *Australopithecus* skull, even when the latter is compared with correspondingly immature skulls of the chimpanzee and gorilla. It is suggested that the anterior margin of the foramen in *Paranthropus* may have been about 5 mm. behind the level tentatively indicated in Broom's fig. 85, Pl. VIII (see his recent monograph). Even with this adjustment, however, it is definitely advanced in position as compared with recent anthropoid apes, for the posterior margin of the occipital condyle is on a transverse level with the posterior margin of the tympanic bone, while in the gorilla and chimpanzee it is considerably behind this level.

The maximum width of the foramen magnum in *Paranthropus* is estimated to be about 30 mm., which is considerably above that found in adult male chimpanzee skulls (the maximum of which in a series of five skulls was found not to exceed 25 mm.), and only equalled by the foramen magnum of large male gorilla skulls. In the *Plesianthropus* type skull, the maximum transverse diameter of the foramen magnum was probably 24 mm. In the *Paranthropus* skull, part of the occipital bone behind the foramen magnum is preserved, and is found to extend back in an approximately horizontal plane for about 25 mm. This again, is in marked contrast with the post-occipital element in the chimpanzee and gorilla, in which it slopes upwards rather abruptly from the posterior border of the foramen magnum, especially in the adult.

Muscular ridges. The development of powerful muscular ridges in the temporal and nuchal regions of the anthropoid ape skull is a well-recognized character. It seems important, therefore, to emphasize their relatively weak development in the Australopithecine fossils. Part of the roof of the skull of the type specimen

of *Plesianthropus* is available (and also a negative cast, in the limestone matrix, of the top of the skull), including the bregma and surrounding area. From this fragment it can be said quite definitely that no temporal ridge was present within at least 33 mm. of the median line. But the skull is that of an adult in which the last molar tooth had erupted and already shown signs of wear. In adult male chimpanzees and gorillas, the temporal ridges have commonly fused in the mid-line at this time (and, in any case, so far as my own observations go, are always within 20 mm. of the mid-line). Indeed, in one instance, fusion was found to have occurred in a chimpanzee skull in which the last molar tooth was still unerupted on one side. In another chimpanzee skull in which the last molar was in process of eruption, the temporal ridge was found to extend up to 14 mm. from the mid-line, and in a female gorilla skull in which the last molar had erupted (but without showing any sign of wear), the ridge had reached to within 10 mm. of the mid-line. It thus appears that the temporal muscle in *Plesianthropus* must have been relatively poorly developed, a condition undoubtedly related to the small size of the incisors and canine teeth. It has already been noted that the mechanism of the temporo-mandibular articulation in the Australopithecinae resembles that of man, and this is associated with a remarkably flat wear of the molar teeth clearly due to rotatory movements of the mandible. It may be inferred, therefore, that the pterygoid musculature must have been well developed, and, indeed, this is attested by the rather large size of the pterygoid fossa (so far as this can be estimated from portions of the fossa preserved in the skulls of *Australopithecus* and *Paranthropus*). The supra-occipital portion of the *Plesianthropus* skull which has been found is very fragmentary, but it makes clear another important point—that (as Broom has emphasized) it lacked powerful nuchal crests of the type found in the adult male gorilla and chimpanzee. In the *Paranthropus* skull the lateral extension of the nuchal crest into the mastoid region is preserved, and this part of the crest appears to be rather powerfully developed. However, at the posterior margin of the fragment, that is, about 30 mm. behind the level of the posterior margin of the auditory meatus, the ridge is approximately at the level of the upper margin of the zygomatic arch (in the horizontal position of the skull), whereas in the adult male gorilla and chimpanzee, in which it slopes upwards and backwards rather sharply, it is at this point considerably higher. The relatively low position of the lateral part of the nuchal crest in *Paranthropus* confirms the evidence provided by the *Plesianthropus* skull that the nuchal musculature was less powerfully developed in the Australopithecinae than in the modern large apes. This, indeed, is no doubt to be expected from a consideration of the difference in the relative position of the foramen magnum (vide supra) which, again, is related to the poise of the head.

Cranial sutures. Broom describes and figures a spheno-parietal contact at the pterion in the *Australopithecus* skull, and the identification of this sutural pattern on the original specimen is reasonably certain. It has an important bearing on the possibilities of a relationship with the recent African apes, for

Ashley-Montagu (1933) found a true spheno-parietal contact in only three chimpanzee skulls out of 240 (1·2%) and in only three gorilla skulls out of 285 (1·1%). In the *Paranthropus* skull, the bone in the region of the pterion is, unfortunately, not sufficiently well preserved to permit delineation of the sutural pattern.

The sutures on the top of the type *Plesianthropus* skull are shown with great clarity in the bone fragment itself, on the negative cast of the roof of the skull, and also on the endocranial cast. It is a matter of considerable interest that the upper part of the coronal suture and the anterior half of the sagittal suture are still completely patent, though in this individual the last molar had completed its eruption and, indeed, is already slightly worn. In the recent apes the sutures of the cranial vault close very early, commencing at about the time of the eruption of the second molar (Krogman, 1930). It is apparent, therefore, that the closure of the vault sutures was delayed in *Plesianthropus*, presumably indicating a more prolonged growth period than is found in the modern large apes.

The general character of the Australopithecine skull. The facial part of the skull of *Australopithecus* is excellently preserved, and the natural endocranial cast permits a satisfactory reconstruction of the general contour of the calvarium, including certain features of the skull base. The type skull of *Plesianthropus* is sufficiently complete (except for the mandible) to justify a reconstruction of the intact skull with reasonable accuracy. The skull fragments of *Paranthropus* include the facial and temporal regions, some important components of the basis cranii, and most of the mandible. It is thus possible, from this material, to enumerate with considerable confidence all the outstanding features of the Australopithecine skull. In general appearance the combination of massive jaws with a small brain case comparable in size with that of a large chimpanzee or of a gorilla suggests to the inexperienced or uncritical eye a creature closely related to the modern large apes. However, those who are well acquainted with primate anatomy will recognize some highly significant differences. These are to be seen in the degree of alveolar prognathism (conditioned by the relative size of the incisor and canine teeth), the contour of the supra-orbital and frontal regions, the appearance in lateral view of the orbits (particularly the recession of the lateral margin), the forward position of the auditory meatus, temporo-mandibular joint and foramen magnum, the shape and disposition of the zygomatic bone (including the level of its temporal process), the details of the glenoid cavity, the contour of the aveolar borders of the maxilla and mandible, the late closure of the sutures of the cranial vault, and the relatively weak development of muscular ridges. In all these features, the Australopithecinae show a very definite approach to *Homo*, and in many of them the detailed resemblances seem to be sufficiently remarkable to exclude the idea of parallelism as a possible explanation.

ENDOCRANIAL CASTS

One of the remarkable features connected with the discovery of the Australopithecine fossils is the degree of perfection of portions of natural endocranial casts which have been found (see, for example, Pl. 1, fig. 3). As is well known, an almost complete natural cast was found in association with the *Australopithecus* skull. Remains of three similar casts have been recovered from Sterkfontein, of which the first, almost complete, belonged to the type skull of *Plesianthropus*. All these casts have been described and discussed in great detail by Dr G. W. H. Schepers (see Broom & Schepers, 1946). An examination of the original specimens shows that the convolutional markings which are preserved on the casts are much more distinct and sharply defined than in the endocranial casts of the recent anthropoid apes or modern man. It is important that this should be made clear, for some anatomists (including the present writer) have expressed doubts regarding the feasibility of delineating more than a very few sulci from such casts. On the other hand, it seems certain that the identity of some of the sulci which Schepers has described and figured (particularly those in the parietal and occipital areas) are open to question, since the corresponding impressions on the casts are rather too vague and indefinite for certain identification. As the result of a careful study, it was possible to confirm the practical certainty of Schepers's identifications of the following sulci (referred to by the numbers employed in the diagrams in his monograph).

Australopithecus. 11, 7, 8, 4, 5(*i*), 13, 16*pt*, 17, 18*p*, 23 (posterior half), 25 (posterior horizontal part), 27, anterior extremity of lateral sulcus. There appears to be some doubt as to the identification of the anterior occipital sulcus (26 in Schepers's diagrams), but that the groove on the cast which apparently corresponds to the upper part of the sulcus is not an artefact seems to be assured by the fact that it is crossed by an uninterrupted impression of the parieto-temporal branch of the middle meningeal vessels.

Plesianthropus type 1.* 4 (anterior part), 5(*i*), 5*s*, 7, 9, 11, 14 (over a small extent bounded in front by a local eminence which appears to correspond to the 'arm eminence' described in the endocranial casts of chimpanzees—see Le Gros Clark, Cooper & Zuckerman, 1936), anterior extremity of the lateral sulcus, anterior portions of 23 and 27 (but not the terminal parts descending on the temporal pole.)

Plesianthropus type 2. 1, 4, 5(*i*), 7, 8, 11, 12, 13, anterior extremity of lateral sulcus.

Plesianthropus type 3. It seems doubtful whether any sulci can be certainly delineated on the surface of this imperfect cast.

It should be emphasized that Schepers's identification of the sulci not mentioned as confirmed in the foregoing lists is not entirely denied. These sulci may have been present in the brain precisely as he has indicated them in

* It should be noted that Schepers refers to the three endocranial casts of *Plesianthropus* as types 1, 2 and 3.

his diagrams, but a critical examination of the original casts suggests that the details of their pattern can only be surmised rather inconclusively.

Apart from the size and general shape of the endocranial casts of the fossil Australopithecinae, there are two features of particular phylogenetic importance to which reference should be made—the degree of convolutional complexity and the position (or presence) of the sulcus lunatus (or simian sulcus). The first impression obtained by an examination of the original casts of *Australopithecus* and *Plesianthropus* is that the sulci on the frontal lobe are, in fact, rather more numerous and close-set than they are in the brains of modern anthropoid apes. Whether this impression will stand the test of quantitative methods can only be decided by careful comparative studies, for the convolutional pattern in the modern large apes shows considerable variability. Certainly the sulci in the occipital region of *Australopithecus* appear to be more complex than they are commonly found to be in the gorilla and chimpanzee and, indeed, it is partly for this reason that the identification of a sulcus lunatus has presented some difficulty. Dart claimed that this sulcus in the *Australopithecus* brain was close to the occipital pole (in a relative position in which it may occasionally be found in the brain of modern man), and this identification is supported by Schepers (who labels the sulcus 18 in his diagrams). If this is the case, it must be taken to indicate a remarkable expansion of the parietal cortex by comparison with the brain of modern apes. Dart's interpretation has been criticized by other anatomists who claim that the sulcus lunatus must have been placed much more anteriorly, i.e. in the position of the typical simian sulcus of a chimpanzee or gorilla. Now, the markings of the convolutions on a great part of the occipital lobe of *Australopithecus* are particularly distinct, so that their pattern can here be delineated with certainty. A close study seems to make it fairly clear that, if the sulcus lunatus is farther forward than the depression numbered 18 in Schepers's diagram, it must be at least as far anterior as the depression marked 26 (for between 18 and 26 the convolutions are disposed in an approximately antero-posterior direction, and they are clearly enough marked on the cast to exclude the possibility of a transversely disposed sulcus lunatus in this position). But a sulcus lunatus in the position of the depression marked 26 would be much farther forward (relatively to the distance between the frontal and occipital poles of the cerebral hemisphere) than in any modern ape, and would indicate an area striata of an extent and complexity beyond reasonable expectation. Thus it must be inferred that a sulcus lunatus, if present in typical form, must have been at least as far back as sulcus 18. On the other hand, the impression on the cast which has been interpreted as sulcus 18 is very ill-defined, and may simply be related to an elevation of bone along the line of the lambdoid suture and not to a cortical sulcus at all. Whichever interpretation may be correct, the evidence provided by the cast can certainly be taken to indicate that the brain belonging to the *Australopithecus* skull did not possess a lunate sulcus of the typical simian type. Unfortunately, the endocranial casts of the occipital region of the skull in the *Plesianthropus*

material do not provide certain information about the number and disposition of the sulci in the occipital lobes of the brain and thus do not permit confident inferences regarding the presence of a sulcus lunatus.

Of the cranial capacity of the Australopithecine fossils, there is little which can be added to the information already available. The endocranial casts of *Australopithecus* and *Plesianthropus* (type 1) are sufficiently complete to justify an estimate of their total volume. Dart's original estimate for *Australopithecus* was 520 c.c., and Zuckerman's estimate of 500 c.c. is sufficiently close to be taken as confirmatory. According to Zuckerman's data on the skull growth of chimpanzees (1928), the cranial capacity of *Australopithecus* would probably have increased to about 570 c.c. in the adult (or rather more if Dart's somewhat higher estimate is accepted). This compares with a range of 325–500 c.c. reported for male chimpanzees and 415–655 c.c. for male gorillas. Thus it appears that the cranial capacity of *Australopithecus* certainly exceeded that of the chimpanzee, and reached the upper level recorded for adult male gorillas. On the other hand, all the evidence (provided by limb bones) so far available indicates that the Australopithecine apes were by no means heavily built, and it may, therefore, be inferred with a fair degree of probability that in the adult *Australopithecus* the brain-weight/body-weight ratio must have exceeded that of the male gorilla. The cranial capacity of the type specimen of *Plesianthropus* is reasonably estimated by Schepers to have been 435 c.c., which is well within the range of variation in chimpanzees. The limb-bone fragments of *Plesianthropus* which have been discovered indicate a relatively small body weight by comparison with *Homo*, but give no indication that it was any less than the average body weight of chimpanzees. Thus, so far as the evidence of cranial capacity is concerned, there is no reason to suppose that in its brain development *Plesianthropus* had surpassed the chimpanzee.

Unfortunately, only a small portion of the brain case of *Paranthropus* has so far been found, and from this Schepers has estimated that the cranial capacity must have been about 650 c.c. It will probably be generally agreed that it is hardly possible to arrive at even an approximate estimate in this case, since only the temporal region of one side of the calvarium is available for the reconstruction of the whole endocranial cast. On the other hand, an inspection of the original material does give grounds for supposing that the cranial capacity must have been larger than in the *Australopithecus* specimen, for the side wall of the cranium rises almost vertically for at least 41 mm. above the level of the root of the zygomatic process of the temporal bone (and a close examination of the original specimen gives no reason for supposing that there has been any post-mortem deformation of the bone which might account for this orientation). In this remarkable feature the skull of *Paranthropus* differs very conspicuously from that of the modern anthropoid apes in which the side wall begins to slope towards the mid-line almost as soon as it rises above the level of the zygomatic process. There can be little doubt, therefore, that the skull of *Paranthropus* must have been markedly hypsicephalic, and, though the absence of the occipital

region of the calvarium precludes an accurate estimate of the degree of brachycephaly, it is reasonable to infer that the cranial capacity was probably large in relation to the width of the skull.

A brief reference may be made to the size of the internal carotid artery and transverse venous sinus in the fossil material, since the indirect evidence they provide of the volume of the blood supply to the brain confirms the general impression that, while the brain may have been rather larger in proportion to the body weight than in the modern anthropoid apes, it definitely fell within the simian range. In the base of the skull of the type specimen of *Plesianthropus* there is a sharply defined natural cast of the carotid canal. This measures 3·5 mm. in diameter, and is thus considerably less than the sectional area of the carotid canal in man (see Cunningham, 1902). The diameter of the impression of the transverse sinus in *Australopithecus* is 5·0 mm. and in *Plesianthropus* 5·5 mm., and these correspond well with that of modern apes.

Considering the fidelity with which some of the surface features of the cerebral hemispheres have been reproduced in the natural endocranial casts of the Australopithecinae, it is to be regretted that so little information can be derived from them concerning the mental functions of these extinct creatures. The fact is, however, that attempts during recent years to correlate the convolutional markings of the endocranial casts of primates with the relative development of different functional areas of the cerebral cortex have met with very little success. In the first place the sulcal impressions on the endocranial casts of the higher primates are usually too indefinite to allow the certain identification of more than a few of them. Secondly, even the study of the surface of the actual brain itself does not permit the delineation of cortical areas with an accuracy sufficient for comparisons on a quantitative basis, for it must be admitted that the precise relationship of the boundaries of most of these areas to neighbouring sulci shows a great deal of variability. It is true that if the central sulcus and the lunate sulcus can be definitely identified on an endocranial cast, they allow an accurate definition of the boundary between the motor and general sensory areas of the cortex and of the anterior boundary on the lateral surface of the cerebral hemisphere of the visual cortex (area striata). Unfortunately, however, these sulci (the only sulci on the lateral surface of the hemisphere which maintain a fairly constant relation to functional areas of the cerebral cortex) are but rarely indicated on endocranial casts. Thus it is not even possible to draw any legitimate inference from the study of the Australopithecine casts regarding elementary cortical functions, and, even if the whole sulcal pattern could be clearly delineated, no deductions could properly be made regarding such complex functions as speech, manual skill and general intelligence. From the general size and proportions of the endocranial casts it might be suggested that the mental powers of the Australopithecines were probably not much superior to those of the chimpanzee and gorilla, but this is the limit of legitimate speculation on the basis of such crude evidence. However, there is indirect evidence of quite a different nature which certainly

makes it probable that the Australopithecinae were equipped with an intelligence definitely in advance of that possessed by the modern large apes. This evidence consists of a series of baboon skulls (*Parapapio izodi*) discovered at the Taungs site, most of which show small and localized depressed fractures in the cranial vault. Dart has taken the view that these injuries must have been caused by a blow of some weapon used by *Australopithecus* in hunting and killing these animals. This interpretation appears to be reasonable, and the present writer is certainly able to confirm that the appearance of these depressed fractures on the baboon skulls is as Dart has described them.

THE DENTITION

The anatomical characters of the Australopithecine dentition have been discussed in great detail by Dart (1934), Broom & Schepers (1946), and Gregory & Hellman (1939) as the result of studies of the original fossil material, and also by Adloff (1932), Abel (1931), Senyürek (1941) and Montandon (1939) on the basis of casts and photographs. There is little that can be added to the information already published. The present writer was able to confirm all the descriptive details reported by Broom and Dart, and was much impressed with the remarkably human characters apparent in the milk dentition and in the attritional wear of the permanent premolars and molars. There is no doubt that these human appearances obtrude themselves much more forcibly on the eye in the examination of the original teeth than in the examination of plaster casts (however excellent and accurate the latter may be). The special importance of the milk dentition lies in the consideration that, if the human characters of the adult Australopithecine dentition are after all merely secondary developments, i.e. the result of a convergent evolution from a more typically simian dentition of the Dryopithecine type, then the 'primitive' simian features might at least be expected to manifest themselves in the milk dentition. However, this is by no means the case, as was first demonstrated by Dart in *Australopithecus*. The milk dentition of *Paranthropus* is now also known in the greatest detail from the beautifully preserved teeth which Dr Broom discovered on the Kromdraai site not more than 4 ft. from the spot where the adult *Paranthropus* skull was obtained. These teeth reproduce the same general features (but with some differences in detail) already described in the milk dentition of *Australopithecus*. The incisors and canine teeth are, in their diminutive size, beyond the range of variation recorded for the corresponding deciduous teeth of the modern large apes (Remane, 1921), and the first deciduous molar appears to be entirely human in its shape, proportions and cusp pattern (see Pl. 1, fig. 6). The *Australopithecus* jaw belongs to a relatively older child than the immature *Paranthropus* jaw, for in the former the first molar had almost completed its eruption. A point of some considerable interest lies in the extent to which the deciduous molars in the *Australopithecus* specimen have been worn down by attrition. It would be difficult to express such a feature quantitatively, but such an exposure of the dentine by wear has not been observed by the present writer

in the milk molars of modern anthropoid apes of a corresponding age. According to the information at present available (see Schultz, 1935), in the chimpanzee the milk molars have completed their eruption by the seventeenth month and the first permanent molars come into position as early as the end of the third year. Thus, there is a comparatively short interval of about 19 months between the two periods of eruption during which the milk molars are exposed to attrition. In man the milk molars have usually erupted before the end of the second year, while the first permanent molar does not come into position till the sixth year. In this case, therefore, the interval between the two events is much longer (perhaps as much as 4 years). The degree of attrition of the milk molars in *Australopithecus* suggests rather strongly that in this extinct genus the eruption time of the first permanent molar may have been delayed as it is in man. If this inference is sound, it serves to confirm the implication of the delayed closure of the sutures of the cranial vault in *Plesianthropus* (vide supra), i.e. that the growth period of the Australopithecinae was prolonged beyond that of the modern large apes. Such a conclusion is of considerable significance, for the prolongation of the growth period is highly characteristic of the human family.

Attention may further be drawn to a very significant feature concerning the relative degree of wear of the permanent teeth in the Australopithecinae. In the type mandible of *Paranthropus* the third lower molar had evidently only recently come into position, for it is practically unworn. Yet the first molar is already planed down to an approximately flat surface with exposure of the dentine, while in the second molar the minor sulci have disappeared and the main cusps are worn smooth (Pl. 1, fig. 4). Such a degree of differential attrition in the three molars at this early stage of maturity is rarely if ever found in the recent anthropoid apes. Indeed, as pointed out by Schultz, noteworthy attrition of the teeth is unusual in primates with an incompletely erupted permanent dentition. In man, however, it is not uncommon, partly, no doubt, in relation to the much longer interval between the eruptions of the molar teeth in the human dentition. According to Schultz's final estimates, the interval between the eruption of the first and second molar, and the second and third molar, is in each case $3\frac{1}{2}$ years in the chimpanzee and $6\frac{1}{2}$ years in man. In other words, the length of time during which each molar is exposed to wear before the next comes into place is almost doubled in man. Thus the degree of differential wear of the lower molars in *Paranthropus* is strongly suggestive of an adolescent period similar in length to that of modern man (and considerably longer than that of the large apes of to-day). The maxillary teeth of the type skull of *Plesianthropus* provide corroborative evidence for this inference. In this specimen the roots of the third upper molar are not yet fully formed (Broom & Schepers, 1946). Yet in the first molar all the cusps are worn down to a considerable degree, and the second molar also shows quite marked signs of wear. Another feature of particular interest is the degree of attrition of the premolar teeth. In the recent anthropoid apes the premolars do not erupt till *after* the second molar teeth; consequently they show very little evidence of

attrition before the last molar comes into full use. In man the premolars commonly erupt during the eleventh or twelfth year, before the appearance of the second molars; they may thus be in use for 8 or 9 years before the completion of the eruption of the last molars, and sometimes (particularly in primitive races living on a rough diet) show a considerable degree of wear at a period when the last molars are as yet practically unworn. In the *Paranthropus* mandible, and rather more conspicuously in the upper dentition of *Plesianthropus* type skull (see Pl. 1, fig. 2), the premolars already show very evident signs of wear. In the *Plesianthropus* specimen, the degree of wear of the premolars relative to that of the second molar is particularly instructive. It appears almost certain, from this indirect evidence, that in the sequence of the appearance of the permanent teeth, as well as in the length of the intervals between their eruption, the Australopithecinae must have resembled man and not the recent anthropoid apes.

That the dental anatomy of the Australopithecinae as a whole is far more human than simian is sufficiently attested by the conclusions of all those authorities who have made a serious study of the fossil teeth. It is instructive to record these conclusions in summary form. Dart (1934) stated that there is no really significant feature in the dentition of *Australopithecus* that can be termed simian, and that 'if any single tooth in this dentition had been found separately, it would unquestionably have been called a human tooth'. Broom (Broom & Schepers, 1946) sums up his comprehensive survey of all the available Australopithecine teeth by saying that 'the dentition of both the milk and the permanent sets agrees remarkably closely with the dentition of man, and only differs in retaining a few more primitive characters and in being a little larger in most cases'. Gregory (1930), in a comparative study of the teeth of *Australopithecus*, drew up a list of resemblances to, or differences from, other hominoids; out of twenty-six characters he found that in twenty the *Australopithecus* teeth are transitional to, or nearer to, primitive man, while in only three characters are they nearer to the chimpanzee or gorilla. In a subsequent intensive study of all the Australopithecine teeth then available, Gregory & Hellman (1939) were led to agree with Broom that 'in South Africa there once lived apes which had almost become men'. Abel (1931), in a critical examination of the Australopithecine dentition, thought the teeth showed some signs of specialization, but he recognized many of the remarkable hominoid features. For example, he noted that little difference is to be found in the comparison of the first milk molar with that of recent hominids. Adloff (1932) was more definite in his conclusions, for he emphatically affirms: 'Das Gebiss von *Australopithecus* ist aber rein menschlich und lasst den Schluss zu, dass *Australopithecus* kein Anthropoide sondern ein Hominide ist.' Senyürek (1941) concluded from his studies of the teeth that the Australopithecines 'are nearer to the primitive hominids than to the apes', and Montandon (1939) expressed exactly the same opinion. As the result of his study of the original material, the present writer is able to add his support to all these conclusions by expressing

the opinion that, on the basis of the dentition alone, there can be no question of any close affinity of the Australopithecinae with the modern anthropoid apes, or even with the Dryopithecinae. In other words, if only the evidence of the teeth were to be taken into account, the allocation of these fossil creatures to the Hominidae rather than to the Pongidae would seem a logical necessity.

THE LIMB BONES

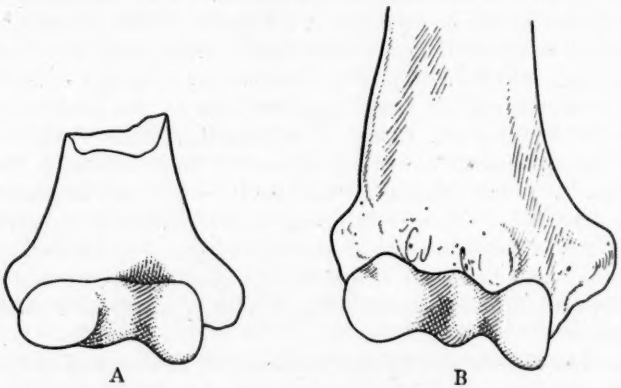
At Sterkfontein and Kromdraai some important fragments of limb bones have been found which have been ascribed to *Plesianthropus* and *Paranthropus*. Those which we shall consider in this section include the lower end of a left femur and a right os capitatum from Sterkfontein, and the lower end of a right humerus, upper end of a right ulna and a right talus from Kromdraai. The striking fact which has emerged from Dr Broom's studies is that all these bones show remarkably human characteristics; indeed, the lower end of the humerus and the femoral fragment, if considered alone and without reference to the circumstances of their recovery, would probably be classified as belonging to *Homo sapiens*. It may be questioned, therefore, whether the limb bones are correctly to be associated with the skulls and teeth of *Plesianthropus* and *Paranthropus*. Are they, for example, the limb bones of some hominid form which lived contemporaneously with the Australopithecinae, or which, though found in the same kind of bone breccia and on the same site, are really derived from a geological horizon of later date? The available evidence is against such suppositions. They are all heavily mineralized like the skull bones. Dr Broom has affirmed that the humeral and ulnar fragments and the talus were found close together in the same bone breccia which contained the type skull of *Paranthropus* and within 1 ft. of it. The ulnar and humeral fragments articulate in close conformity with each other and thus almost certainly represent the component parts of the right elbow joint of the same individual. The talus also belongs to the right side. All these bones are identifiable as the bones of a large hominoid, and no remains of a large hominoid have been found at the site apart from *Paranthropus*. Also, no other remains of any large limb bones have been discovered at the Kromdraai site which could be attributed to *Paranthropus*. Lastly, in spite of an extensive search through great quantities of breccia, during which many skulls, teeth and bones of lower mammals have been found, no trace has been seen of human skulls and teeth, nor any signs of human habitation. The intrinsic evidence of the limb bones themselves provides the final justification for their association with *Paranthropus*, for, though predominantly human in their general appearance, they have quite special characteristics of their own. As will be seen below, the humerus and the talus both present exceptional features, and in the case of the talus there is an interesting combination of simian and human characters which harmonizes well with the same sort of combination provided by the skull, brain and teeth of *Paranthropus*.

The capitate bone and lower end of the femur ascribed to *Plesianthropus* were not found in direct association with the remains of the skull and teeth, but were

derived from the limestone matrix at the Sterkfontein site where (as Broom states) the remains of not less than three separate skulls of *Plesianthropus* have been discovered. As with the Kromdraai specimens, they are both clearly bones of a large hominoid, and no remains of any fossil hominoids apart from *Plesianthropus* have been found at the Sterkfontein site. There is certainly no trace of human skulls and teeth, or of human occupation. The capitate bone shows a combination of human and simian characters which is in harmony with the skull and brain of *Plesianthropus*. The lower end of the femur is adapted for a completely erect posture as in man, but also shows peculiarities of its own. Thus, although the human appearance of these two bones, particularly the femur, might raise a doubt as to the correctness of their association with skulls and brains which are so simian in their general proportions, the available evidence is entirely in favour of this association. The climatological evidence is also pertinent to this question, for it indicates that the Australopithecinae were certainly not forest-living creatures like the recent anthropoid apes. They must, therefore, have been adapted to a completely terrestrial life, which would have required the type of lower limb indicated by the femoral fragment discovered at Sterkfontein. Lastly, as at Kromdraai, many skulls, teeth and bones of lower mammals have been exposed in the breccia from the Sterkfontein site, but among this mass of material there is no sign of other types of hominoid limb bones which could belong to *Plesianthropus* excepting these. Taking all this evidence into careful account, the present writer finds no reason to doubt the Australopithecine nature of the large limb bones so far discovered at Kromdraai and Sterkfontein and ascribed to *Paranthropus* and *Plesianthropus* by Dr Broom.

The humerus. The lower end of the right humerus of *Paranthropus* is quite well preserved except for a small flake off the anterior surface of the capitulum. It shows a very close resemblance to the humerus of *Homo sapiens* and none of the distinctive features found in the recent anthropoid apes (Text-fig. 3). The lateral supracondylar ridge is moderately defined as in man, and as it descends it curves gently forwards to the summit of the lateral epicondyle. On the lower part of the latter is a triangular facet of quite human appearance for the attachment of the extensor muscles of the forearm. The pronator ridge on the medial border of the lower end of the shaft is weakly developed. The medial epicondyle is more slender and pointed than is commonly seen in man, and its projecting apex inclines very slightly backwards, as well as medially and downwards. It shows a facet for the attachment of the flexor muscles of the forearm, which is equivalent in size to that seen on a human humerus. The coronoid fossa is well marked and relatively rather deep, while the radial fossa is indicated by a very shallow depression above the medial half of the capitulum. The olecranon fossa corresponds in its extent and depth to that of man, and thus contrasts with the rather deep excavation seen in the chimpanzee and gorilla (associated with the powerful development of the olecranon process of the ulna). There is no perforation of the olecranon fossa as is usually found in

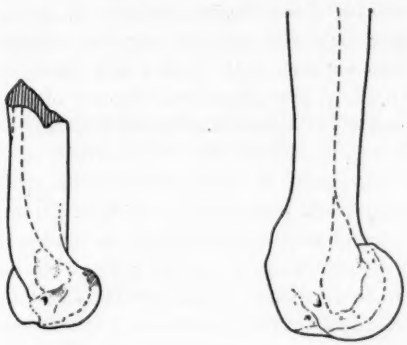
gorillas (80 %) and also in some chimpanzees. The triceps and brachialis surfaces of the lower end of the shaft of the humerus are smooth, and their contours are quite similar to those of man. Broom suggests that the curvature of the articular surface of the capitulum shows certain features which may distinguish it from the humerus of modern man, but, if this should prove to be the case, they are probably too slight to be of great significance. On the other hand, the position of the capitulum in relation to the axis of the lower end of the shaft of the humerus (particularly as seen in lateral view) is not set quite so far forward as it normally is in *Homo sapiens*. This relation of the lower articular end to the shaft of the humerus is much more pronounced in the trochlear surface, as seen both from the lateral and medial views of the humerus (Text-figs. 4, 5). In the human (and more so in the simian) humerus, the trochlea is



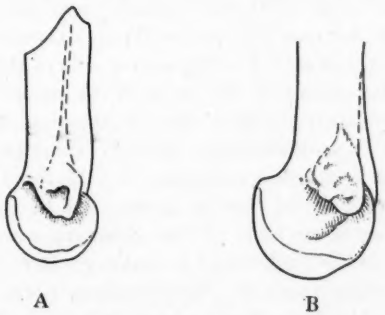
Text-fig. 3. The lower end of the right humerus of *Paranthropus* (A) seen from in front, compared with that of an adult male chimpanzee (B). × ⅓.

set well forward in relation to the shaft, and especially in relation to the medial epicondyle. In *Paranthropus*, on the other hand, the transverse central axis of the trochlea lies approximately within the line of the long axis of the lower end of the shaft of the humerus. The significance of this alinement of the trochlea is not at all clear. Mechanically it must mean some limitation of the power of flexion (since the leverage in the flexor action of the biceps and brachialis muscles would be somewhat diminished) and a capacity for hyper-extension at the elbow joint to a degree not usually possible in recent anthropoid apes or man. The only other feature of the humeral fragment which calls for mention is the presence of a deep pit about 2 mm. in diameter, evidently a vascular foramen, behind the lateral epicondyle. Around this foramen the bone is slightly roughened. It is suggested that this is the result of a mild localized periostitis (probably due to trauma) which has led to increased vascularization and the enlargement of one of the vascular foramina which are usually found in this position.

The ulna. The upper end of the right ulna (which consists of little more than the olecranon process and the upper two-thirds of the trochear notch) was found in close proximity to the lower end of the right humerus of *Paranthropus*, and since they articulate together in close conformity, there is no doubt (as Broom has also stated) that they belong to the same individual. Like the humeral fragment, the ulnar fragment appears to correspond in all its main



Text-fig. 4. The lower end of the right humerus of *Paranthropus* (A) seen from the lateral aspect, compared with that of an adult male chimpanzee (B). $\times \frac{2}{3}$.



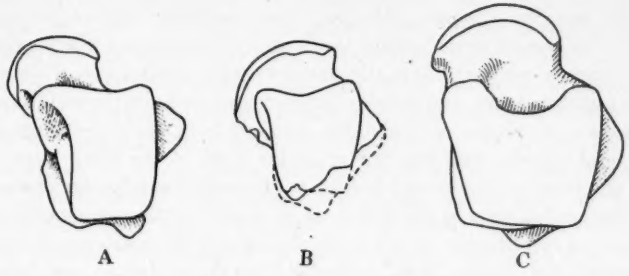
Text-fig. 5. The lower end of the right humerus of *Paranthropus* (A) seen from the medial aspect, compared with that of an adult male chimpanzee (B). $\times \frac{2}{3}$.

features to the human bone. The curvature of the trochlear notch and the prominence of the 'beak' of the olecranon process (so far as this is preserved) are not quite so marked as they usually are in *Homo*. On the upper aspect of the olecranon process the rim of the articular surface (for the attachment of the joint capsule) is preserved in its lateral third and is separated by a narrow well-defined groove from a smooth bursal area which closely corresponds in its extent with that of the human ulna. The area for the attachment of the triceps is

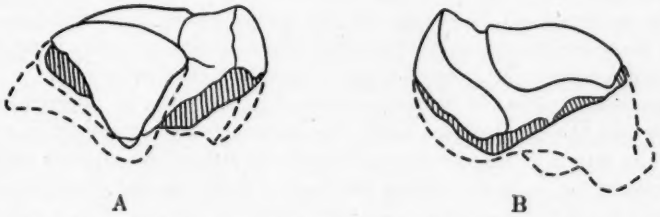
relatively small and but slightly roughened. In all these characters, the *Paranthropus* ulna contrasts strongly with that of the anthropoid apes, which shows a massive development of the olecranon process with particularly prominent ridges on its medial and lateral aspects demarcating the surfaces for the attachment of the flexor digitorum profundus and anconeus muscles. These features are, of course, related to the powerful development of the arms in the recent anthropoid apes for use in brachiation.

The talus. The talus of *Paranthropus* presents a number of remarkable features which together appear to indicate a general construction of the bone somewhat intermediate between that of man and the lower hominoids. The whole bone is very small, as is evident from the size of the superior articular surface (for articulation with the lower end of the tibia). The width of this at its anterior margin is 20·5 mm., which corresponds closely with that of an adult chimpanzee foot. In Europeans the same dimension is stated to have a range of 31–37 mm., and in female Japanese 28–30 mm. (Adachi, 1905). In two Bushman skeletons which have been examined, the measurements were 23 and 25 mm. It may also be of interest to record that in the talus of a European child aged 12 years in this department, the width is 23 mm.

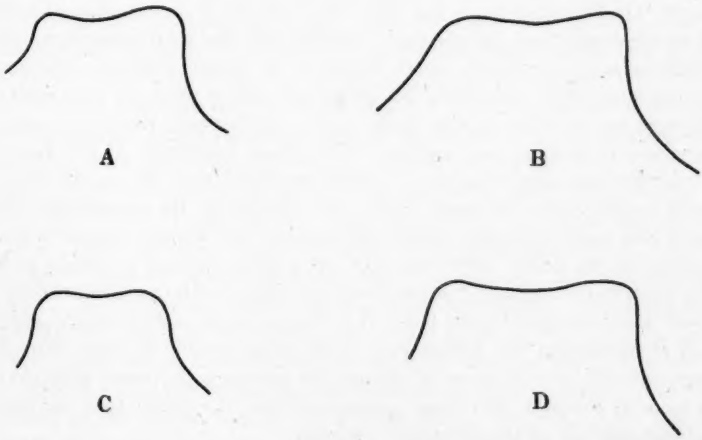
The superior articular facet of the *Paranthropus* talus resembles man in being relatively broad and also in its even curvature from side to side (Text-figs. 6, 8). In the gorilla and chimpanzee, the surface in transverse section is somewhat flattened medially, and laterally slopes gradually upwards to the lateral border. This asymmetrical contour is related to a different orientation of the subtalar articular facets (see Morton, 1926), and is possibly associated with the fact that the load line of the femur and tibia passes through their medial condyles and presumably is transmitted rather to the medial side of the body of the talus. In man (as also in *Paranthropus*) the shape of the superior surface evidently indicates a more even distribution in the transmission of body weight. The lateral fibular facet is somewhat less strongly curved than in the recent anthropoid apes, and is also less extensive. In the fossil talus, it should be noted, the lower margin of this facet at its apex can be made out close to the lower fractured surface of the bone. In the chimpanzee and gorilla, the maximum vertical extent of the fibular facet is always greater than the width of the superior articular surface taken at a level midway between its anterior and posterior borders. In the *Paranthropus* talus, as in man, it is less. The medial facet (for the medial malleolus) is quite similar in contour and extent to that of man, and its articular surface is approximately vertical. In the gorilla and chimpanzee this surface slopes medially to a marked extent (Text-fig. 8), and in the chimpanzee it is excavated anteriorly to form a conspicuous cup-shaped socket which engages and locks with the anterior margin of the medial malleolus, so limiting movement at full dorsiflexion. The neck of the fossil talus is rather short, but its unusual relative width tends to give this character a somewhat exaggerated appearance. The wide neck of the talus is associated with an extent of the articular surface (for the navicular bone) on the 'head' of the



Text-fig. 6. The dorsal aspect of the right talus of a male chimpanzee (A), *Paranthropus* (B) and a female Japanese (C). $\times \frac{2}{3}$.



Text-fig. 7. Lateral (A) and medial (B) views of the right talus of *Paranthropus*. The reconstructed portions are indicated by interrupted lines. Natural size.



Text-fig. 8. The contours of transverse sections through the talus in the plane of the maximum extension of the medial and lateral facets. A, chimpanzee; B, gorilla; C, *Paranthropus*; D, man. Natural size.

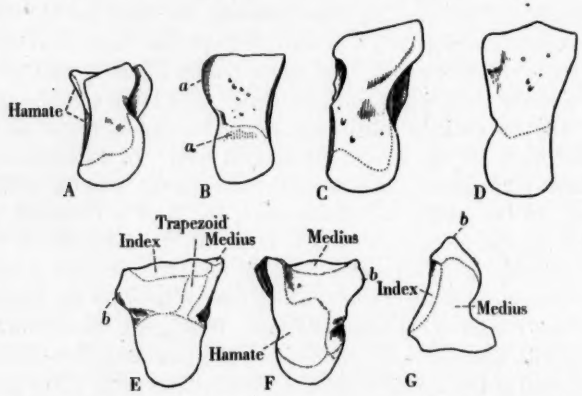
bone which is remarkable (particularly when it is considered in relation to the size of the bone as a whole). Medially this navicular surface projects to a distance of at least 8 mm. beyond the level of the plane of the medial tibial articular facet (when the talus is orientated with the central axis of the superior facet in a sagittal plane), and in this character it reproduces the condition found in the gorilla and chimpanzee and differs strongly from the modern human talus. In its lateral extent, however, the articular head of the fossil talus reaches towards the level of the lateral border of the superior articular surface, well beyond the central axis of the latter, as in man, and thus contrasts with the typical simian condition in which it hardly extends laterally beyond the level of the central axis. Lastly, it will be noted from the accompanying figures that in the fossil bone the type of curvature of the head corresponds with the human and not with the chimpanzee talus. In the latter the head is of much more hemispherical form and permits of greater freedom of movement at the subtalar joints in eversion and inversion. In the gorilla where, in adaptation to a heavier body weight and more terrestrial habits, greater stability is required, the articular surface of the head is more broadly curved as in man.

From a consideration of these anatomical details, it may be inferred that in *Paranthropus* the body of the talus was constructed for stability in weight bearing, as shown by the contour and relative width of the superior articular facet, and for the rapid alternating movements of flexion and extension of the ankle joint required for walking, and particularly for running. This is evidenced by the contour and orientation of the facets for the malleoli, which with the superior articular surface form a composite 'trochlear' articulation of much greater efficiency as a pure hinge-joint mechanism than in the modern anthropoid apes. On the other hand, the slope and contour of the lateral and medial facets in the gorilla and chimpanzee, together with the relative narrowness of the superior articular facet, clearly provide for more complex movements (involving some degree of side-to-side deflection) and at the same time limit the weight-bearing stability of the ankle joint, particularly during attempted movements of walking and running. The broad curvature of the head of the *Paranthropus* talus evidently provides for stability in the transmission of the body weight to the fore part of the foot in standing. Its remarkable extent medially and laterally would presumably permit the transference of a major component of the body weight directly forwards as in man or would permit it to be deflected medially to the extent which is found in the modern anthropoid apes with their divergent great toes. It is thus perhaps permissible to suggest that in *Paranthropus* the hallux may have been capable of some degree of divergence which would allow of its use for grasping purposes, and that it could also be brought into close alinement with the other toes for use in the human posture of standing and walking.

The capitate bone. A number of features of great interest in this bone have been already detailed by Dr Broom. As he has pointed out, it is unusually small and has a very human appearance. This is shown particularly in the

proportions of the bone, for it is shorter and broader than that of the chimpanzee and gorilla (in which the bone is elongated in conformity with the elongation of the hand as a whole), and also in the shape of the distal articular surface. In the chimpanzee, the distal articular surface is divided into several concave facets, separated by intervening ridges, which articulate with the rather complex articular surfaces at the base of the third metacarpal. In the gorilla the distal articular surface of the capitate is less complex, but it is distinctly convex from side to side in its dorsal part, for articulation with a concavity on the base of the third metacarpal bone. In man, the articular surface is much more flattened, curving over towards the lateral aspect of the bone at its dorsal border in conformity with the styloid process of the third metacarpal; consequently, the distal border of the human capitate bone, viewed from the dorsal aspect, is markedly oblique. In *Plesianthropus* the facet for the third metacarpal is but gently curved as it is in man—except that there is no very definite deflection for a styloid process. The distal border of the dorsal surface thus appears as a relatively straight transverse edge (as it often is in the chimpanzee capitate). Another striking feature mentioned by Broom is the disposition of the articular facet for the second metacarpal bone. In *Plesianthropus*, as in man, this facet is elongated and is as much on the distal as on the medial surface of the bone. In the gorilla and chimpanzee, the facet is relatively small and broadly rectangular in contour, and it faces directly laterally on the lateral surface of the bone, being separated from the facet for the third metacarpal by a sharp acute-angled border. There is a long narrow facet for the trapezoid bone near the dorsal border of the lateral surface. Here again the resemblance is to some extent with man, for in the chimpanzee this facet is quite small and placed at the distal end of the lateral surface of the capitate, while in the gorilla there is no contact between the capitate and the trapezoid bones. No facet for articulation with the fourth metacarpal appears to be present, but this facet is also absent in about 10 % of Europeans. A feature of some importance in the *Plesianthropus* capitate bone is the presence of a pronounced concavity in the middle of the non-articular portion of the lateral surface of the bone. Being situated opposite a slight concavity on the medial surface, this gives the bone a somewhat 'waisted' appearance when viewed from its palmar aspect (Text-fig. 9A). In the chimpanzee and gorilla, this lateral concavity is a still more pronounced feature, and presumably serves to attach a strong interosseous ligament. In the human capitate bone, on the other hand, the lateral surface is usually broadly convex, so that the whole bone, viewed from its palmar aspect, appears much more robust. The articular surfaces for the scaphoid and trapezoid bones on the lateral aspect of the os capitatum are also much more extensive in the human than in the *Plesianthropus* bone and, here again, the latter shows a significant simian feature. Lastly, attention should be given to a curious simian feature represented by a blunt pointed process projecting forward from the distal end of the anterior (palmar) surface of the fossil bone (see Text-fig. 9E, G). This process is well developed in the chimpanzee, and

presumably serves for the attachment of strong intercarpal ligaments. A survey of all the characters of the *Plesianthropus* capitate indicates that these are predominantly human. Whether the radial concavity and the ligamentous process on the anterior surface of the bone are within the range of variation found in man is hardly possible to affirm without a very extensive comparative study of the human carpus. However, in spite of the examination of all the material available to the present writer (European and otherwise), it has not been possible to match these simian features in the capitate bone of modern man. It thus seems legitimate to conclude that the fossil bone (as Broom also implies) occupies a somewhat intermediate position in its morphological characters. If this is so, of course, it adds very strong support to the natural inference that



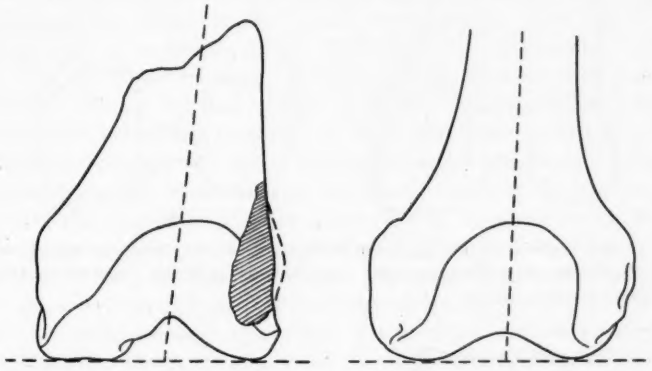
Text-fig. 9. The right capitate bone of *Plesianthropus* (A, B, E–G) compared with that of a chimpanzee (C, D). Natural size. A and C, palmar aspect; B and D, dorsal aspect; E, lateral aspect; F, medial aspect; G, distal aspect. Slight abrasions of the surface of the fossil bone are indicated at *a*. The ligamentous process on the distal part of the palmar surface (mentioned in the text) is labelled *b*.

the bone does actually belong to *Plesianthropus*. In size the *Plesianthropus* bone is equivalent to that of a Bushman and very much smaller than that of an adult chimpanzee. Probably it is legitimate to infer that the movements between the capitate and the adjacent bones of the hand were less free in *Plesianthropus* than in Europeans, but much more free than in the hand of the modern anthropoid apes.

The femur. The distal extremity of a left femur discovered in the lower cave at Sterkfontein has been ascribed by Broom to *Plesianthropus*. There can be little doubt that this allocation is correct. The femoral fragment is clearly that of a large hominoid, and intensive and prolonged searches in the Sterkfontein deposits have failed to reveal the remains of any large hominoid other than *Plesianthropus*. Moreover, the small size of the femur is in harmony with the small size of the capitate bone which was also found in the Sterkfontein matrix

and which, as already indicated, is almost certainly to be referred to *Plesianthropus*. In its anatomical details, however, the bone shows a resemblance to the femur of *Homo* which is so close as to amount to practical identity.

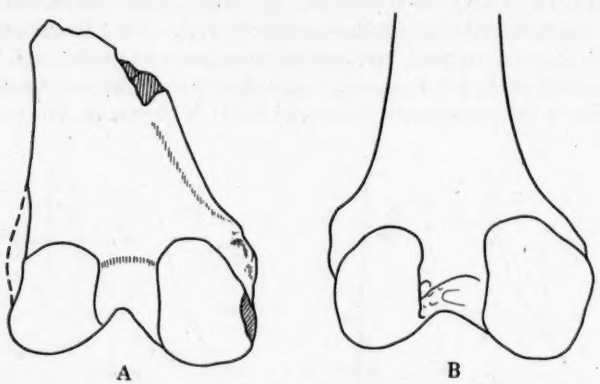
The size of the bone is remarkably small. The bicondylar width (measured by the method described by Parsons, 1913–14) is 56·5 mm., which is considerably below the lowest measurement recorded by Parsons for adult English femora of either sex. On the other hand, it corresponds to the bicondylar width of a European child of 12 years (as shown by a comparison with material in this department) or to that of an adult male chimpanzee. In four Bushman skeletons examined, the measurement was found to range from 53 to 63·5 mm. The lower end of the shaft of the femur, however, is relatively to the bicondylar width more robust than in the chimpanzee, European child, or Bushman. The width of the



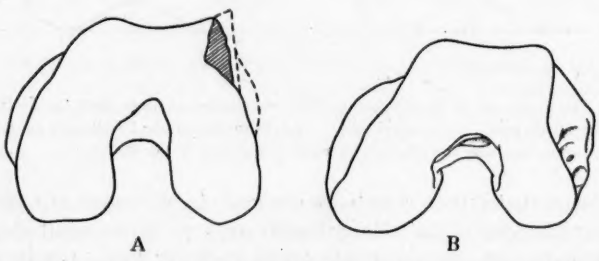
Text-fig. 10. The lower end of the left femur of *Plesianthropus* (A) seen from in front, compared with that of an adult chimpanzee (B). $\times\frac{3}{4}$. Note the marked obliquity of the shaft in *Plesianthropus*, and also the relatively greater robustness of the shaft.

shaft can be estimated with reasonable accuracy to be 32 mm. at a distance of 6·5 cm. from the level of the tibial articular surfaces. In an adult chimpanzee the corresponding measurement was found to be 26 mm., and in the only Bushman skeleton in which the bicondylar width did not exceed that of the *Plesianthropus* femur, 24·5 mm. The obliquity of the shaft of the fossil bone, that is to say the angle between the axis of the shaft and the vertical axis in the standing position, makes a strong contrast with that of the femora of modern anthropoid apes. The angle can be approximately estimated by placing the tibial articular surfaces on a flat surface and measuring the angle of inclination of the axial line extending from the centre of the preserved part of the shaft to the mid-point of the anterior end of the intercondylar notch. By this method, it can be shown (see Text-fig. 10) that the angle of obliquity is at least 7°. This compares with an average of 10° recorded by Parsons for normal male English

femora (with a range of 4–17°). In the African anthropoid apes, the obliquity of the femoral shaft is very slight, the average for the gorilla being (according to Pearson & Bell, 1919) 1·8° and for the chimpanzee – 0·1°. It may be inferred, therefore, that in the erect standing position adopted by *Plesianthropus* the femur sloped downwards and medially as in man. The contour of the patellar surface is in conformity with this conclusion. For while in the anthropoid apes



Text-fig. 11. The lower end of the left femur of *Plesianthropus* (A) seen from behind, compared with that of an adult chimpanzee (B). ×⅔. Note the relatively larger size of the medial condyle in the chimpanzee.



Text-fig. 12. The lower end of the left femur of *Plesianthropus* (A) seen from below, compared with that of an adult chimpanzee (B). ×⅔.

this surface is broad, shallow, and evenly curved, thus permitting considerable freedom of lateral movement between the patella and the femur, in *Plesianthropus* it is relatively deeper, and laterally slopes rather abruptly into a prominent lip. It should be mentioned that the summit of this lip is actually missing in the fossil bone, but its position can be reconstructed with fair accuracy. If the condyles are viewed from below and compared with those of the modern anthropoid apes (Text-fig. 12), the contrast is striking. The shape and disposition of the condyles are again typically human, and are separated

by a relatively narrow intercondyloid notch. The latter is prolonged forwards to an unusual extent, and at its anterior end presents a notch which is an exaggeration of a similar notch often to be observed in human femora. This notch, as pointed out by Siddiqi (1934), is related to the pressure of the anterior cruciate ligament of the knee joint in full extension, and appears to indicate, therefore, that the joint could be habitually sustained in this typically human position in *Plesianthropus*. A comparison of views of the posterior aspect of the fossil femur and that of the chimpanzee serves to emphasize another interesting point. In the modern anthropoid apes, the load line of the body in the standing position passes through or medial to the medial condyle of the femur (Walmsley, 1933). In relation to this fact the medial condyle presents a much larger articular surface than the lateral condyle. In modern man, the load line in the erect standing position (with the knees together) passes through the lateral condyle which thus becomes more important for weight bearing; in the human femur, therefore, the medial condylar articular surface is relatively less strongly developed. In the *Plesianthropus* femur, the proportionate sizes of the two condyles are quite similar to those of *Homo*.

In addition to the observations just recorded, the following descriptive points may also be referred to. The lateral epicondyle is almost entirely missing from the femoral fragment. However, near the lateral margin of the lateral condyle a portion of the posterior half of the groove for the popliteus tendon remains. The medial epicondyle is well preserved, and shows a distinct facet for the medial head of the gastrocnemius muscle. The adductor tubercle is small, but forms quite a well-defined pointed process, and leading down to it is a rather strongly developed supracondylar ridge. The popliteal surface is smooth and slightly concave. About 14 mm. above the posterior extremity of the lateral condyle, and extending laterally across the lateral supracondylar ridge, is a low rough eminence which presumably marks the attachment of the plantaris muscle. A strong rounded ridge separates the intercondylar fossa from the popliteal surface, which in its definition and general appearance is quite similar to that of *Homo sapiens*. The lateral and medial walls of the intercondylar fossa are hollowed out but show no definite impressions of the attachment of the cruciate ligaments. It has been mentioned above that the intercondylar notch is prolonged forwards to an unusual extent, in association with the exaggerated development of the impression related to the anterior cruciate ligament. Broom has already referred to this forward extension and states that he has been unable to match it in femurs of Kafirs, Europeans, Bushmen, Amerindians and Australian natives. A further examination of large numbers of femora in this department and elsewhere has also failed to demonstrate a precise parallel in modern man.

A survey of the anatomical details of the *Plesianthropus* femur, and a critical examination of the accompanying diagrams, will make it quite clear that the bone is constructed almost entirely on the human plan. In other words it is mechanically adapted for standing, walking and running in the erect

position. It thus offers a complete contrast to the femur of the modern anthropoid apes. In the latter, the hind-limbs may be used as temporary struts on which these animals can occasionally balance themselves in an approximately upright position, but they do not permit of progression in the fully erect position characteristic of man.

CONCLUSIONS

Those who are acquainted with the previous accounts of the Australopithecinae which have been published from time to time by Dart and Broom will recognize that much of the present communication has been concerned with 'underlining' and amplifying statements already made by these South African authorities. Such additional information as may have been presented only serves to reinforce their general conclusions that the Australopithecinae represent by far the most important discoveries which have so far been made in the field of human palaeontology. These fossil creatures were at a simian level of evolution in regard to the size of the brain and the general proportions of the skull, but in the details of their anatomical structure they present no evidence of any close affinity to the recent anthropoid apes. On the other hand, the resemblances which they show to man in the morphological features of the skull, dentition and limb bones are so remarkable that their zoological relationship to the Hominidae can hardly be doubted. It is particularly interesting to note that the evidence derived from the study of different parts of the skeleton is reciprocally confirmatory. Thus, the nature of the wear of the permanent teeth leads to the inference that the temporo-mandibular joint was constructed on the human pattern, and this is borne out by an examination of the skull. The position of the occipital condyles indicates a poise of the head and a bodily posture which approximates to that of man, and this is confirmed by an examination of the limb bones. Further, the climatological evidence leads to the inference that the Australopithecinae were well adapted for terrestrial (and not for arboreal) life, and this is reflected in the conformation of the lower end of the femur. Lastly, the size and proportion of the brain, as well as the massive jaws, the large size of the molar and premolar teeth, and certain features of the reduced canine teeth, are definitely ape-like characters which, in combination with the more advanced characters of the skull and dentition, indicate a morphological status in some degree intermediate between the simian and human levels of evolution. In conformity with this is the remarkable combination of simian and human characters presented by the talus and os capitatum, to which reference has been made in this paper.

The anatomical study of the Australopithecine fossils necessarily raises the question whether their hominid characters betoken an ancestral relationship to man, or only a collateral relationship. So far as purely morphological criteria are concerned, there seems to be no serious objection to the conception of an ancestral relationship, for the fossil material so far available shows no evidence of any gross specialization which would necessarily preclude this.

Indeed, the major importance of these fossils lies in the fact that they conform so closely in their anatomical structure to theoretical postulates for an intermediate phase of human evolution, such as had been based on the indirect evidence of comparative anatomy. However, the place occupied by the Australopithecinae in human phylogenesis will be precisely determined only when more adequate palaeontological and geological data are available. At present the evidence for assessing the antiquity of the limestone deposits in which the South African fossils were found is based on faunal associations and is still inconclusive. We may, however, note Dr Broom's tentative estimate (Broom & Schepers, 1946) that the Sterkfontein deposits are of upper Pliocene date (or earlier) and that the Taungs deposits may even be attributed to the middle Pliocene. If it should be established, as the results of further systematic excavations, that the Australopithecine fossil material is no older than the early Pleistocene or late Pliocene, it must probably be assumed that, at the most, these extinct hominoids represent the little modified survivors of the ancestral stock from which, at a still earlier date, the line of human evolution originated.

Certain inferences of a general nature may be drawn from a consideration of the morphological features of the Australopithecinae. In the first place, it now seems clear that in human phylogenesis the evolution of the limb structure proceeded at a more rapid rate than that of the brain, since in these fossils the limb skeleton approximates to that of *Homo* while the brain volume hardly surpasses the simian level. This conclusion fits in well with the evidence provided by the remains in Java and China of *Pithecanthropus*, for in this early fossil hominid the limb bones appear to be quite similar to those of modern man while the skull, teeth and brain still show very primitive characters. The Australopithecine material also supports inferences drawn from the indirect evidence of comparative anatomy that many of the diagnostic characters of the recent large anthropoid apes such as the powerful canines and the large incisors, the sectorial form of the first lower premolar, the exaggerated development of the supra-orbital torus, and the construction of the powerful brachiating arms, are to be regarded as aberrant specializations peculiar to these apes and not as primitive features to be sought for in human ancestry. If such reasoning is sound, however, it must also apply (at least in part) to the extinct dryopithecine apes, for these had already to some degree developed the typical simian specializations of the dentition. Hence, it appears very doubtful whether the conception of a 'dryopithecine phase' in human evolution is any longer tenable. Finally, the more detailed knowledge of Australopithecine anatomy which is now available demands a careful reconsideration of the taxonomic status of these extinct hominoids. It seems clear that while in their cerebral development, and therefore in the *general* proportions of the skull, they represent a level of evolution corresponding to that of the large anthropoid apes, they show no structural evidence of close relationship to the latter. On the contrary, the advanced characters which are already very evident in their skull

dentition and limb bones indicate their position in the phylogenetic radiation of the Hominidae rather than the Pongidae.

I wish to express my gratitude to my friends Prof. Raymond Dart and Dr Robert Broom for the very generous hospitality which they gave me during my visit to their laboratories. They placed their valuable fossil material completely at my disposal, with every facility for its study, and they also provided special arrangements for visiting the sites of their outstanding discoveries.

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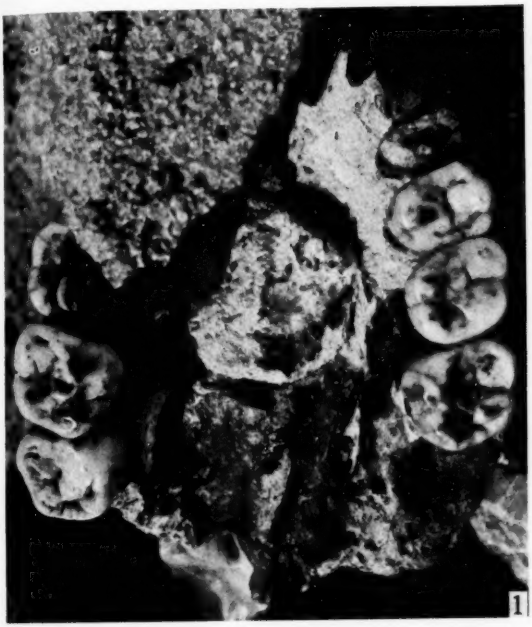

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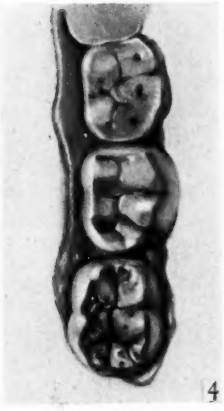

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EXPLANATION OF PLATE

Fig. 1. Upper teeth and palate of *Paranthropus*. $\times\frac{2}{3}$ approx. Since this photograph was taken, three more crowns of the upper teeth were found. Photograph lent by Dr R. Broom.

Fig. 2. Occlusal view of the left maxillary teeth of the type specimen of *Plesianthropus*. $\times\frac{2}{3}$ approx. Note the type and degree of wear shown in the premolar teeth. Photograph lent by Dr R. Broom.

Fig. 3. Natural endocranial cast of the type specimen of *Plesianthropus* lying in contact with the base of the skull. $\times\frac{1}{3}$ approx. After this photograph was taken, some of the facial skeleton was exposed by removal of the limestone matrix. In the photograph can be seen the supra-orbital ridges (*a*), the natural casts of the frontal air sinuses (*b*), and the lateral margin of the left orbit (*c*). Photograph lent by Dr R. Broom.

Fig. 4. Occlusal view of the right lower molar teeth of *Paranthropus*, drawn from a cast. $\times\frac{10}{9}$ approx. Note the degree of differential attrition in the three molars.

Fig. 5. The first right lower molar of *Paranthropus* from a photograph of the original specimen lent by Dr Broom, showing the type of wear. $\times 3$ approx.

Fig. 6. Photographs (natural size) of a cast of the deciduous lower dentition of the right side (and the incompletely erupted first permanent molar) of *Paranthropus*. *a*, occlusal view; *b*, lateral view. Note particularly the relatively small size of the lateral incisor and the canine, and the human appearance of the first deciduous molar.

ADDENDUM

Since this paper was sent to the press, further remarkable discoveries have been made by Dr Broom at Sterkfontein (see *Nature*, Vol. 159, 1947, pp. 602, 672 and 809). The newly discovered material (which represents the remains of at least eight individuals) includes a practically complete skull of *Plesianthropus*, the upper jaw of a young adult, the upper jaw of an immature individual with the milk dentition, an almost complete mandible, and portions of limb bones. It will necessarily take some time before all this new material can be fully developed from the limestone matrix in which it is embedded, but certain points have already emerged from the preliminary descriptions published by Dr Broom. These confirm the inferences already drawn regarding the contours of the frontal and occipital regions of the skull, the relatively low position of the nuchal crest, the recession of the lateral orbital margin and the relatively forward position of the occipital condyles. Other features of interest include the wide contact between the lacrimal and ethmoid bones in the medial wall of the orbit, the relatively wide extension of the lesser wings of the sphenoid with a probable spheno-ethmoidal contact in the anterior fossa of the skull, and the sharp under-cut anterior margins of the middle cranial fossa formed by the posterior borders of the lesser wings of the sphenoid. This last feature is evidently related to a pronounced degree of flexion of the basi-cranial at the junction of the pre- and post-sphenoid elements.

NOTICES OF BOOKS RECEIVED

Anatomy and Physiology for Students of Physiotherapy, Occupational Therapy and Gymnastics. By C. F. V. Smout, M.D., M.R.C.S., L.R.C.P., and R. J. S. McDowall, M.D., D.Sc. 2nd ed. (Pp. viii+470; 318 figs.; demy 8vo; 30*s.* net.) London: Edward Arnold and Co.

A well-produced publication 'planned in strict accordance with the Syllabus of the Chartered Society of Physiotherapy'. The first 333 pages deal with Anatomy, the next 127 with Physiology and the remaining 10 pages contain a Glossary.

This new edition contains a chapter on 'Levers and Leverage', by Miss Weir, of the Anstey Physical Training College, Birmingham. A new section on the demonstration of muscles in the living body has been added to the Anatomy part, and a chapter on 'Bacteria and Infection' has been added to the Physiology part which, as a whole, has been considerably enlarged.

Anatomie Comparée du Cerveau. By M. Friant, D.Sc. (Pp. 123; 117 figs.; pott 8vo; 450 Frs.) Paris: Collection Orion; Éditions Prisma.

An elementary account, illustrated by line drawings and photographs, of the external appearances of the cerebral hemispheres in man and various mammals. Forty-seven of the figures show the appearance of the human cerebral hemispheres at various stages of foetal life.

Research in Holland: Experimental Embryology. By M. W. Woerdeman, M.D. and Chr. P. Raven, Ph.D. (Pp. xi + 132; 40 illustrations; demy 8vo; paper; 13*s.* net.) New York and Amsterdam: Elsevier Publishing Company, Inc. Distributors, Cleaver-Hume Press Ltd. London.

The book, no. 10 of a series of twenty-three monographs dealing with the progress of research in Holland during the war, describes research on experimental embryology carried out by a number of workers at the Anatomical-Embryological Laboratory of the University of Amsterdam, and at the Laboratory of General Zoology of the State University of Utrecht.

Subjects dealt with at Amsterdam include experiments on amphibian eggs concerning: determination of the lens-primordium; determination of the polarity of the ectoderm; limb-induction and neural crest; influence of trivalent arsenic on the development of amphibian eggs; and influence of carcinogenic carbohydrates on the development of amphibian larvae.

From Utrecht there comes an interesting account of the development of the pond snail (*Limnaea stagnalis*) and of experiments on amphibian eggs, particularly *Xenopus laevis*.

The Integrative Action of the Nervous System. By Sir Charles Sherrington, O.M. (Pp. xxiv+433; frontispiece, 85 figs.; demy 8vo; 25*s.* net.) Cambridge University Press.

A new and entirely reset edition of the work published originally under the same title by Scribner's of New York in 1906, and based on lectures delivered at Yale University by Sir Charles Sherrington during his tenure of the Silliman Memorial Lectureship. Now published with portrait, curriculum vitae and bibliography of the writings of the author. The present edition is edited by Prof. Samson Wright, under the aegis of the Physiological Society. Sir Charles Sherrington has written a 12-page foreword.